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(54) Title: NOVEL NEMATODE-ACTIVE TOXINS AND GENES WHICH CODE THEREFOR (57) Abstract This invention concerns genes or gene fragments which have been cloned from novel <i>Bacillus thuringiensis</i> isolates which have nematocidal activity. These genes or gene fragments can be used to transform suitable hosts for controlling nematodes.		

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NOVEL NEMATODE-ACTIVE TOXINS AND
GENES WHICH CODE THEREFOR

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Cross-Reference to a Related Application

This is a continuation-in-part of co-pending application Serial No. 07/693,018, filed on May 3, 1991; which is a continuation-in-part of Serial No. 07/565,544, filed on August 10, 1990; which is a continuation-in-part of application Serial No. 084,653, filed on August 12, 1987. This is also a continuation-in-part of application Serial No. 07/830,050, filed on January 31, 1992.

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Background of the Invention

Regular use of chemicals to control unwanted organisms can select for chemical resistant strains. This has occurred in many species of economically important insects and has also occurred in nematodes of sheep, goats, and horses. The development of chemical resistance necessitates a continuing search for new control agents having different modes of action.

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In recent times, the accepted methodology for control of nematodes has centered around the drug benzimidazole and its congeners. The use of these drugs on a wide scale has led to many instances of resistance among nematode populations (Prichard, R.K. et al. [1980] "The problem of anthelmintic resistance in nematodes," Austr. Vet. J. 56:239-251; Coles, G.C. [1986] "Anthelmintic resistance in sheep," In *Veterinary Clinics of North America: Food Animal Practice*, Vol 2:423-432 [Herd, R.P., eds.] W.B. Saunders, New York). There are more than 100,000 described species of nematodes.

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The bacterium *Bacillus thuringiensis* (*B.t.*) produces a δ -endotoxin polypeptide that has been shown to have activity against a rapidly growing number of insect species. The earlier observations of toxicity only against lepidopteran insects have been expanded with descriptions of *B.t.* isolates with toxicity to dipteran and coleopteran insects. These toxins are deposited as crystalline inclusions within the organism. Many strains of *B.t.* produce crystalline inclusions with no demonstrated toxicity to any insect tested.

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A small number of research articles have been published about the effects of delta endotoxins from *B. thuringiensis* species on the viability of nematode eggs. Bottjer, Bone and Gill (Experimental Parasitology 60:239-244, 1985) have reported that *B.t. kurstaki* and *B.t. israelensis* were toxic in vitro to eggs of the nematode *Trichostrongylus colubriformis*. In addition, 28 other *B.t.* strains were tested with widely variable toxicities. The most potent had LD₅₀ values in the nanogram range. Ignoffo and Dropkin (Ignoffo, C.M. and Dropkin, V.H. [1977] J. Kans. Entomol. Soc. 50:394-398) have reported that the thermostable toxin from *Bacillus thuringiensis* (beta exotoxin) was active against a free-living nematode, *Panagrellus redivivus* (Goodey); a plant-parasitic nematode, *Meloidogyne incognita* (Chitwood); and a fungus-feeding nematode, *Aphelenchus avena* (Bastien). Beta exotoxin is a generalized cytotoxic agent with little or no

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specificity. Also, H. Ciordia and W.E. Bizzell (Jour. of Parasitology 47:41 [abstract] 1961) gave a preliminary report on the effects of *B. thuringiensis* on some cattle nematodes.

At the present time there is a need to have more effective means to control the many nematodes that cause considerable damage to susceptible hosts. Advantageously, such effective means would employ biological agents.

Brief Summary of the Invention

The subject invention concerns novel toxins active against nematodes. A further aspect of the invention concerns genes coding for nematocidal toxins. The subject invention provides the person skilled in this art with a vast array of nematocidal toxins, methods for using these toxins, and genes that code for the toxins.

One aspect of the invention is the discovery of two generalized chemical formulae common to a wide range of nematocidal toxins. These formulae can be used by those skilled in this art to obtain and identify a wide variety of toxins having the desired nematocidal activity. The subject invention concerns other teachings which enable the skilled practitioner to identify and isolate nematode active toxins and the genes which code therefor. For example, characteristic features of nematode-active toxin crystals are disclosed herein. Furthermore, characteristic levels of amino acid homology can be used to characterize the toxins of the subject invention. Yet another characterizing feature pertains to immunoreactivity with certain antibodies. Also, nucleotide probes specific for genes encoding toxins with nematocidal activity are described.

In addition to the teachings of the subject invention which define groups of *B.t.* toxins with advantageous nematocidal activity, a further aspect of the subject invention is the provision of specific nematocidal toxins and the nucleotide sequences which code for these toxins.

One aspect of the of the subject invention is the discovery of two groups of *B.t.*-derived nematode-active toxins. One group (CryV) is exemplified by the gene expression products of PS17, PS33F2 and PS63B, while the other group (CryVI) is exemplified by the gene expression products of PS52A1 and PS69D1. The organization of the toxins within each of the two groups can be accomplished by sequence-specific motifs, overall sequence similarity, immunoreactivity, and ability to hybridize with specific probes.

The genes or gene fragments of the invention encode *Bacillus thuringiensis* δ -endotoxins which have nematocidal activity. The genes or gene fragments can be transferred to suitable hosts via a recombinant DNA vector.

Brief Description of the Sequences

SEQ ID NO. 1 discloses the DNA of 17a.

SEQ ID NO. 2 discloses the amino acid sequence of the toxin encoded by 17a.

SEQ ID NO. 3 discloses the DNA of 17b.

SEQ ID NO. 4 discloses the amino acid sequence of the toxin encoded by 17b.

SEQ ID NO. 5 is the nucleotide sequence of a gene from 33F2.

SEQ ID NO. 6 is the amino acid sequence of the protein expressed by the gene from 33F2.

SEQ ID NO. 7 is the nucleotide sequence of a gene from 52A1.

5 SEQ ID NO. 8 is the amino acid sequence of the protein expressed by the gene from 52A1.

SEQ ID NO. 9 is the nucleotide sequence of a gene from 69D1.

SEQ ID NO. 10 is the amino acid sequence of the protein expressed by the gene from 69D1.

SEQ ID NO. 11 is the nucleotide sequence of a gene from 63B.

10 SEQ ID NO. 12 is the amino acid sequence of the protein expressed by the gene from 63B.

SEQ ID NO. 13 is the amino acid sequence of a probe which can be used according to the subject invention.

SEQ ID NO. 14 is the DNA coding for the amino acid sequence of SEQ ID NO. 13.

15 SEQ ID NO. 15 is the amino acid sequence of a probe which can be used according to the subject invention.

SEQ ID NO. 16 is the DNA coding for the amino acid sequence of SEQ ID NO. 15.

SEQ ID NO. 17 is the N-terminal amino acid sequence of 17a.

SEQ ID NO. 18 is the N-terminal amino acid sequence of 17b.

20 SEQ ID NO. 19 is the N-terminal amino acid sequence of 52A1.

SEQ ID NO. 20 is the N-terminal amino acid sequence of 63B.

SEQ ID NO. 21 is the N-terminal amino acid sequence of 69D1.

SEQ ID NO. 22 is the N-terminal amino acid sequence of 33F2.

SEQ ID NO. 23 is an internal amino acid sequence for 63B.

25 SEQ ID NO. 24 is a synthetic oligonucleotide derived from 17.

SEQ ID NO. 25 is an oligonucleotide probe designed from the N-terminal amino acid sequence of 52A1.

SEQ ID NO. 26 is the synthetic oligonucleotide probe designated as 69D1-D.

SEQ ID NO. 27 is the forward oligonucleotide primer from 63B.

30 SEQ ID NO. 28 is the reverse oligonucleotide primer from 63B.

SEQ ID NO. 29 is the nematode (NEMI) variant of region 5 of Höfte and Whiteley.

SEQ ID NO. 30 is the reverse complement primer to SEQ ID NO. 29, used according to the subject invention.

35 SEQ ID NO. 31 is a reverse oligonucleotide primer used according to the subject invention.

SEQ ID NO. 32 is the DNA coding for the primer of SEQ ID NO. 31.

SEQ ID NO. 33 is oligonucleotide probe 33F2A.

SEQ ID NO. 34 is oligonucleotide probe 33F2B.

SEQ ID NO. 35 is a reverse primer used according to the subject invention.

SEQ ID NO. 36 is a forward primer according to the subject invention.

SEQ ID NO. 37 is a probe according to the subject invention.

SEQ ID NO. 38 is a probe according to the subject invention.

SEQ ID NO. 39 is a probe according to the subject invention.

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SEQ ID NO. 40 is a forward primer according to the subject invention.

Detailed Disclosure of the Invention

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The subject invention concerns a vast array of *B.t.* δ -endotoxins having nematocidal activity. In addition to having nematocidal activity, the toxins of the subject invention will have one or more of the following characteristics:

1. An amino acid sequence according to either of the two generic formulae disclosed herein.
2. A high degree of amino acid homology with specific toxins disclosed herein.
3. A DNA sequence encoding the toxin which hybridizes with probes or genes disclosed herein.
4. A nucleotide sequence which can be amplified using primers disclosed herein.
5. A crystal toxin presentation as described herein.
6. Immunoreactivity to an antibody raised to a specific toxin disclosed herein.

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One aspect of the subject invention concerns the discovery of generic chemical formulae which describe toxins having activity against nematodes. Two formulae are provided: one which pertains to nematocidal toxins having molecular weights of between about 45 kDa and 65 kDa, and the other pertains to larger nematocidal proteins having molecular weights from about 65 kDa to about 155 kDa. These formulae represent two different categories of *B.t.* δ -endotoxins, each of which has activity against nematodes. The formula describing smaller proteins describes many CryV proteins, while the formula describing larger proteins describes many CryVI proteins. A description of these two formulae is as follows:

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Generic Formula I. This formula describes toxin proteins having molecular weights from about 65 kDa to about 155 kDa. The first 650-700 amino acids for proteins in excess of about 75 kDa and the entire molecule (for proteins of less than about 75 kDa) have substantially the following sequence:

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1  MOXXXXXXXXPX  BPYNBLOXXP  XZXXXXXXXXXX  OXxxxBXXxE  UXBKXBJJXX
   XOxxxxZXXZ  xXOBXJXBJX  XBXXXXBXYX  XXVUXZLZLB  xxxXXOBPXB
101 ZBXXPBLZBB  BXXBXXXXOx  xxXUXOXLBX  XBOXXBUJBL  DJXLXXXXXX
    XLUXELXXBX  XLXXKXXXXB  XExxBXXHXX  BXXBXXZXXX  KBXXXXBZXX
201 ZBXOXXBXXB  LOEXXXJxxx  LXBPTYXBXO  XMXLXXXXXX  LXXZXOWXXK
    Bxxxxxxxxx  XXXXOLXXXX  XXBKXXLXBY  XXXXXXBBXX  XLXZXZxxZX
301 XXXBXJXXXY  XJXMXXX*LE  BXXXXPOBXP  EXYxxxZZXL  XLXKOKXLBZ
    XBBXXXXXXx  XZBOLXUXXX  XOXXXXXXXX  ZXXXBXXXXJ  JBXKxUBKBY
401 XXXXXXX*XX  *Bx*YXXBX  BUXXXXOXXY  ZXxxxXEPXX  ZXXXXxBXXX
    XPBXXBUXXO  XXOXXXXXXXX  XXOXXXXZXB  *XLxxxxxxx  *BXXKX*XXX

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501 ZXZXZXZ*XX XLXZXXXXXX XXXXXXXXXX XZXXXxxxxx XLBXXXXPXE
 XXXXUXLZXX EXXZxUBXXX ZBPBEKxxOZ XXXXBxxBKE WLUZOXXXXL
 601 ZPZUZXBXB OUXOZZXYXB RCRYOZXXXO XBBBUxBXXZ ZXUPLXXUBX
 BXXOXEXXOX XXXXUXBXXB KZLXXXXXXB xxxxxXxJLPX XBXBXBOUX
 701 ZSSXBXLDKL EBBPBX

Numbering is for convenience and approximate location only.

Symbols used:

A = ala	G = gly	M = met	S = ser
C = cys	H = his	N = asn	T = thr
D = asp	I = ile	P = pro	V = val
E = glu	K = lys	Q = gln	W = trp
F = phe	L = leu	R = arg	Y = tyr

K = K or R

E = E or D

L = L or I

B = M, L, I, V, or F

J = K, R, E, or D

O = A or T

U = N or Q

Z = G or S

X = any naturally occurring amino acid, except C.

* = any naturally occurring amino acid.

x = any naturally occurring amino acid, except C (or complete omission of any amino acids).

Where a stretch of wild-card amino acids are encountered (X(n) or x(n) where n>2), repetition of a given amino acid should be avoided. Similarly, P, C, E, D, K, or R utilization should be minimized.

This formula (hereinafter referred to as Generic Formula I) is exemplified in the current application by the specific toxins 17a, 17b and 63b.

Generic Formula II. This formula describes toxin proteins having molecular weights from about 45 kDa to about 65 kDa. Their primary amino acid structure substantially follows the motif illustrated below:

1 MLBXXXXOBP KHxxxXXXXO XXXXZXXKxx xXZPXXBXXX XXBLLZKXEW
 OXBxOYBXOZ XZLPBUJXXB KXHBXLXXJL XLPXJBXULY JBYXXJKXXX
 101 XWWUXXLXPL BBKXOUJLXX YZBKXOZJXX KKxxZXXJXB UJJBJULXJU
 XXJJOXXXKO XKJBXOKCXL LLKEOJUJYJX OQJBXXXXLX XBLXZXUxxx

201 xxjbxzbxxb uxxlxxbxxx lxxxxzjxzp xxjelljkbj xlxxxlexxl
koeujlekbb bxzbxlzpll zbbbyellel oobxxlxxx jxlxxxljxo
5 301 uxjljkjbkl lzbbuzlxoj ljxbxxuzxx olxbbxklxz lwxxlxxulx
ulkxoxxeb xjxxjxjxlx lelxjoxxxw xxboxeoxxb xluzyxxx
401 (x)ⁿ

^aWhere n = 0-100

The symbols used for this formula are the same as those used for Generic Formula I.

This formula (hereinafter referred to as Generic Formula II) is exemplified in the current application by specific toxins 52A1 and 69D1.

Nematode-active toxins according to the formulae of the subject invention are specifically exemplified herein by the toxins encoded by the genes designated 17a, 17b, 63B, 52A1, and 69D1. Since these toxins are merely exemplary of the toxins represented by the generic formulae presented herein, it should be readily apparent that the subject invention further comprises equivalent toxins (and nucleotide sequences coding for equivalent toxins) having the same or similar biological activity of the specific toxins disclosed or claimed herein. These equivalent toxins will have amino acid homology with the toxins disclosed and claimed herein. This amino acid homology will typically be greater than 50%, preferably be greater than 75%, and most preferably be greater than 90%. The amino acid homology will be highest in certain critical regions of the toxin which account for biological activity or are involved in the determination of three-dimensional configuration which ultimately is responsible for the biological activity. In this regard, certain amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Table 1 provides a listing of examples of amino acids belonging to each class.

Table 1	
Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not significantly detract from the biological activity of the toxin. The information presented in the generic formulae of the subject invention provides clear guidance to the person skilled in this art in making various amino acid substitutions.

Further guidance for characterizing the nematocidal toxins of the subject invention is provided in Tables 3 and 4, which demonstrate the relatedness among toxins within each of the above-noted groups of nematocidal toxins (CryV and CryVI). These tables show a numeric score for the best matching alignment between two proteins that reflects: (1) positive scores for exact matches, (2) positive or negative scores reflecting the likelihood (or not) of one amino acid substituting for another in a related protein, and (3) negative scores for the introduction of gaps. A protein sequence aligned to itself will have the highest possible score—i.e., all exact matches and no gaps. However, an unrelated protein or a randomly generated sequence will typically have a low positive score. Related sequences have scores between the random background score and the perfect match score.

The sequence comparisons were made using the algorithm of Smith and Waterman ([1981] Advances in Applied Mathematics 2:482-489), implemented as the program "Bestfit" in the GCG Sequence Analysis Software Package Version 7 April 1991. The sequences were compared with default parameter values (comparison table: Swgappep.Cmp, Gap weight:3.0, Length weight:0.1) except that gap limits of 175 residues were applied to each sequence compared. The program output value compared is referred to as the Quality score.

Tables 3 and 4 show the pairwise alignments between the indicated amino acids of the two classes of nematode-active proteins CryV and CryVI and representatives of dipteran (CryIV; Sen, K. et al. [1988] Agric. Biol. Chem. 52:873-878), lepidopteran and dipteran (CryIIA; Widner and Whiteley [1989] J. Bacteriol. 171:965-974), lepidopteran (CryIA(c); Adang et al. [1981] Gene 36:289-300), and coleopteran (CryIIIA; Herrnstadt et al. [1987] Gene 57:37-46) proteins.

Table 2 shows which amino acids were compared from the proteins of interest.

Table 2	
Protein	Amino acids compared
63B	1-692
33F2	1-618
17a	1-677
17b	1-678
CryIV	1-633
CryIIA	1-633
CryIA(c)	1-609
CryIIIA	1-644
69D1	1-395
52A1	1-475

Table 3 shows the scores prior to adjustment for random sequence scores.

Table 3									
	63B	33F2	17a	CryIVA	CryIIA	CryIA(c)	CryIIIA	52A1	69D1
63B	1038	274	338	235	228	232	244	154	122
33F2		927	322	251	232	251	270	157	130
17a			1016	240	240	237	249	152	127
CryIVA				950	245	325	326	158	125
CryIIA					950	244	241	151	132
CryIA(c)						914	367	151	127
CryIIIA							966	150	123
52A1								713	350
69D1									593

Note that for each nematode-active protein, the highest score is always with another nematode-active protein. For example, 63B's highest score, aside from itself, is with 17a. Furthermore, 33F2's highest score, aside from itself, is also with 17a.

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Similarly, 52A1 and 69D1 have a higher score versus each other than with the other proteins.

Table 4 shows the same analysis after subtraction of the average score of 50 alignments of random shuffles of the column sequences with the row sequences.

Table 4									
	63B	33F2	17a	CryIVA	CryIIA	CryIA(c)	CryIIIA	52A1	69D1
63B	830	81	130	40	32	42	48	0.1	-8.8
33F2		740	128	66	48	72	85	1.4	-2.9
17a			808	45	45	45	54	-0.8	-5.2
CryIVA				759	54	142	138	5.4	-4.1
CryIIA					755	58	53	-2.3	6
CryIA(c)						728	185	3.1	0
CryIIIA							766	-2.3	-6.9
52A1								566	221
69D1									465

Note that in Table 4 the same relationships hold as in Table 3, i.e., 63B's highest score, aside from itself, is with 17a, and 33F2's highest score, aside from itself, is also with 17a.

Similarly, 52A1 and 69D1 have a better score versus each other than with the other proteins.

Thus, certain toxins according to the subject invention can be defined as those which have nematode activity and either have an alignment value (according to the procedures of Table 4) greater than 100 with 17a or have an alignment value greater than 100 with 52A1. As used herein, the term "alignment value" refers to the scores obtained above and used to create the scores reported in Table 4.

The toxins of the subject invention can also be characterized in terms of the shape and location of crystal toxin inclusions. Specifically, nematode-active inclusions typically remain attached to the spore after cell lysis. These inclusions are not inside the exosporium, as in previous descriptions of attached inclusions, but are held within the spore by another mechanism. Inclusions of the nematode-active isolates are typically amorphous, generally long and/or multiple. These inclusions are distinguishable from the larger round/amorphous inclusions that remain attached to the spore. No *B.t.* strains that fit this description have been found to have activity against the conventional targets—Lepidoptera, Diptera, or Colorado Potato Beetle. All nematode-active strains fit this description except one. Thus, there is a very high correlation between this crystal structure and nematode activity.

The genes and toxins according to the subject invention include not only the full length sequences disclosed herein but also fragments of these sequences, or fusion proteins, which retain the characteristic nematocidal activity of the sequences specifically exemplified herein.

It should be apparent to a person skilled in this art that genes coding for nematode-active toxins can be identified and obtained through several means. The specific genes may be obtained from a culture depository as described below. These genes, or portions thereof, may be constructed synthetically, for example, by use of a gene machine. Variations of these genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as *Bal31* or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which code for active fragments may be obtained using a variety of other restriction enzymes. Proteases may be used to directly obtain active fragments of these toxins.

Equivalent toxins and/or genes encoding these equivalent toxins can also be located from *B.t.* isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the nematode-active toxins of the instant invention which occur in nature. For example, antibodies to the nematode-active toxins disclosed and claimed herein can be used to identify and isolate other toxins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the nematode-active toxins which are most constant and most distinct from other *B.t.* toxins. These antibodies can then be used to specifically identify equivalent toxins with the characteristic nematocidal activity by immunoprecipitation, enzyme linked immunoassay (ELISA), or Western blotting. Antibodies to the toxins disclosed herein, or to equivalent toxins,

or fragments of these toxins, can readily be prepared using standard procedures in this art. The genes coding for these toxins can then be obtained from the microorganism.

A further method for identifying the toxins and genes of the subject invention is through the use of oligonucleotide probes. These probes are nucleotide sequences having a detectable label. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong bond between the two molecules, it can be reasonably assumed that the probe and sample are essentially identical. The probe's detectable label provides a means for determining in a known manner whether hybridization has occurred. Such a probe analysis provides a rapid method for identifying nematocidal endotoxin genes of the subject invention.

The nucleotide segments which are used as probes according to the invention can be synthesized by use of DNA synthesizers using standard procedures. In the use of the nucleotide segments as probes, the particular probe is labeled with any suitable label known to those skilled in the art, including radioactive and non-radioactive labels. Typical radioactive labels include ^{32}P , ^{125}I , ^{35}S , or the like. A probe labeled with a radioactive isotope can be constructed from a nucleotide sequence complementary to the DNA sample by a conventional nick translation reaction, using a DNase and DNA polymerase. The probe and sample can then be combined in a hybridization buffer solution and held at an appropriate temperature until annealing occurs. Thereafter, the membrane is washed free of extraneous materials, leaving the sample and bound probe molecules typically detected and quantified by autoradiography and/or liquid scintillation counting.

Non-radioactive labels include, for example, ligands such as biotin or thyroxine, as well as enzymes such as hydrolases or peroxidases, or the various chemiluminescers such as luciferin, or fluorescent compounds like fluorescein and its derivatives. The probe may also be labeled at both ends with different types of labels for ease of separation, as, for example, by using an isotopic label at the end mentioned above and a biotin label at the other end.

Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid, and, as noted above, a certain degree of mismatch can be tolerated. Therefore, the probes of the subject invention include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein said mutations, insertions and deletions permit formation of stable hybrids with the target polynucleotide of interest. Mutations, insertions, and deletions can be produced in a given polynucleotide sequence in many ways, and these methods are known to an ordinarily skilled artisan. Other methods may become known in the future.

The known methods include, but are not limited to:

- (1) synthesizing chemically or otherwise an artificial sequence which is a mutation, insertion or deletion of the known sequence;
- (2) using a probe of the present invention to obtain via hybridization a new sequence or a mutation, insertion or deletion of the probe sequence; and
- (3) mutating, inserting or deleting a test sequence in vitro or in vivo.

It is important to note that the mutational, insertional, and deletional variants generated from a given probe may be more or less efficient than the original probe. Notwithstanding such differences in efficiency, these variants are within the scope of the present invention.

Thus, mutational, insertional, and deletional variants of the disclosed test sequences can be readily prepared by methods which are well known to those skilled in the art. These variants can be used in the same manner as the instant probes so long as the variants have substantial sequence homology with the probes. As used herein, substantial sequence homology refers to homology which is sufficient to enable the variant to function in the same capacity as the original probe. Preferably, this homology is greater than 50%; more preferably, this homology is greater than 75%; and most preferably, this homology is greater than 90%. The degree of homology needed for the variant to function in its intended capacity will depend upon the intended use of the sequence. It is well within the skill of a person trained in this art to make mutational, insertional, and deletional mutations which are designed to improve the function of the sequence or otherwise provide a methodological advantage.

Specific nucleotide probes useful, according to the subject invention, in the rapid identification of nematode-active genes are

- (i) DNA coding for a peptide sequence whose single letter amino acid designation is "REWINGAN" (SEQ ID NO. 13) or variations thereof which embody point mutations according to the following: position 1, R or P or K; position 3, W or Y; position 4, I or L; position 8, N or P; a specific example of such a probe is "AGA(A or G)T(G or A)(G or T)(A or T)T(A or T)AATGG(A or T)GC(G or T)(A or C)A(A or T)" (SEQ ID NO. 14);
- (ii) DNA coding for a peptide sequence whose single letter amino acid designation is "PTFDPDLY" (SEQ ID NO. 15) or variations thereof which embody point mutations according to the following: position 3, F or L; position 4, D or Y; position 7, L or H or D; a specific example of such a probe is "CC(A or T)AC(C or T)TTT(T or G)ATCCAGAT(C or G)(T or A)(T or C)TAT" (SEQ ID NO. 16).

The potential variations in the probes listed is due, in part, to the redundancy of the genetic code. Because of the redundancy of the genetic code, i.e., more than one coding nucleotide triplet (codon) can be used for most of the amino acids used to make proteins. Therefore different nucleotide sequences can code for a particular amino acid. Thus, the amino acid sequences of the *B.r.* toxins and peptides can be prepared by equivalent nucleotide sequences encoding the same amino acid sequence of the protein or peptide. Accordingly, the subject invention includes such equivalent nucleotide sequences. Also, inverse or complement sequences are an aspect of the subject invention and can be readily used by a person skilled in this art. In addition it has been shown that proteins of identified structure and function may be constructed by changing the amino acid sequence if such changes do not alter the protein secondary structure (Kaiser, E.T. and Kezdy, F.J. [1984] Science 223:249-255). Thus, the subject invention includes

mutants of the amino acid sequence depicted herein which do not alter the protein secondary structure, or if the structure is altered, the biological activity is substantially retained. Further, the invention also includes mutants of organisms hosting all or part of a toxin encoding a gene of the invention. Such microbial mutants can be made by techniques well known to persons skilled in the art. For example, UV irradiation can be used to prepare mutants of host organisms. Likewise, such mutants may include asporogenous host cells which also can be prepared by procedures well known in the art.

The toxin genes or gene fragments exemplified according to the subject invention can be obtained from nematode-active *B. thuringiensis* (*B.t.*) isolates designated PS17, PS33F2, PS63B, PS52A1, and PS69D1. Subcultures of the *E. coli* host harboring the toxin genes of the invention were deposited in the permanent collection of the Northern Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois, USA. The accession numbers are as follows:

	<u>Culture</u>	<u>Repository No.</u>	<u>Deposit Date</u>
15	<i>B.t.</i> isolate PS17	NRRL B-18243	July 28, 1987
	<i>B.t.</i> isolate PS33F2	NRRL B-18244	July 28, 1987
	<i>B.t.</i> isolate PS63B	NRRL B-18246	July 28, 1987
	<i>B.t.</i> isolate PS52A1	NRRL B-18245	July 28, 1987
	<i>B.t.</i> isolate PS69D1	NRRL B-18247	July 28, 1987
20	<i>E. coli</i> NM522(pMYC 2316)	NRRL B-18785	March 15, 1991
	<i>E. coli</i> NM522(pMYC 2321)	NRRL B-18770	February 14, 1991
	<i>E. coli</i> NM522(pMYC 2317)	NRRL B-18816	April 24, 1991
	<i>E. coli</i> NM522(pMYC 1627)	NRRL B-18651	May 11, 1990
	<i>E. coli</i> NM522(pMYC 1628)	NRRL B-18652	May 11, 1990
25	<i>E. coli</i> NM522(pMYC 1642)	NRRL B-18961	April 10, 1992

The subject cultures have been deposited under conditions that assure that access to the cultures will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 CFR 1.14 and 35 USC 122. The deposits are available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

Further, the subject culture deposits will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., they will be stored with all the care necessary to keep them viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposit, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the cultures. The depositor

acknowledges the duty to replace the deposits should the depository be unable to furnish a sample when requested, due to the condition of the deposit(s). All restrictions on the availability to the public of the subject culture deposits will be irrevocably removed upon the granting of a patent disclosing them.

5 The novel *B.t.* genes or gene fragments of the invention encode toxins which show activity against tested nematodes. The group of diseases described generally as helminthiasis is due to infection of an animal host with parasitic worms known as helminths. Helminthiasis is a prevalent and serious economic problem in domesticated animals such as swine, sheep, horses, cattle, goats, dogs, cats and poultry. Among the helminths, the group of worms described as nematodes causes
10 wide-spread and often times serious infection in various species of animals. The most common genera of nematodes infecting the animals referred to above are *Haemonchus*, *Trichostrongylus*, *Ostertagia*, *Nematodirus*, *Cooperia*, *Ascaris*, *Bunostomum*, *Oesophagostomum*, *Chabertia*, *Trichuris*, *Strongylus*, *Trichonema*, *Dictyocaulus*, *Capillaria*, *Heterakis*, *Toxocara*, *Ascaridia*, *Oxyuris*, *Ancylostoma*, *Uncinaria*, *Toxascaris*, *Caenorhabditis* and *Parascaris*. Certain of these, such as
15 *Nematodirus*, *Cooperia*, and *Oesophagostomum*, attack primarily the intestinal tract, while others, such as *Dictyocaulus* are found in the lungs. Still other parasites may be located in other tissues and organs of the body.

 The toxins encoded by the novel *B.t.* genes of the invention are useful as nematicides for the control of soil nematodes and plant parasites selected from the genera *Bursaphelenchus*,
20 *Criconebella*, *Ditylenchus*, *Globodera*, *Helicotylenchus*, *Heterodera*, *Meloidogyne*, *Pratylenchus*, *Radopholus*, *Rotelnchus*, or *Tylenchus*.

 Alternatively, because some plant parasitic nematodes are obligate parasites, genes coding for nematocidal *B.t.* toxins can be engineered into plant cells to yield nematode-resistant plants. The methodology for engineering plant cells is well established (cf. Nester, E.W., Gordon, M.P.,
25 Amasino, R.M. and Yanofsky, M.F., Ann. Rev. Plant Physiol. 35:387-399, 1984).

 The *B.t.* toxins of the invention can be administered orally in a unit dosage form such as a capsule, bolus or tablet, or as a liquid drench when used as an anthelmintic in mammals, and in the soil to control plant nematodes. The drench is normally a solution, suspension or dispersion of the active ingredient, usually in water, together with a suspending agent such as
30 bentonite and a wetting agent or like excipient. Generally, the drenches also contain an antifoaming agent. Drench formulations generally contain from about 0.001 to 0.5% by weight of the active compound. Preferred drench formulations may contain from 0.01 to 0.1% by weight, the capsules and boluses comprise the active ingredient admixed with a carrier vehicle such as starch, talc, magnesium stearate, or dicalcium phosphate.

35 Where it is desired to administer the toxin compounds in a dry, solid unit dosage form, capsules, boluses or tablets containing the desired amount of active compound usually are employed. These dosage forms are prepared by intimately and uniformly mixing the active ingredient with suitable finely divided diluents, fillers, disintegrating agents and/or binders such as starch, lactose, talc, magnesium stearate, vegetable gums and the like. Such unit dosage

formulations may be varied widely with respect to their total weight and content of the antiparasitic agent, depending upon the factors such as the type of host animal to be treated, the severity and type of infection and the weight of the host.

When the active compound is to be administered via an animal feedstuff, it is intimately dispersed in the feed or used as a top dressing or in the form of pellets which may then be added to the finished feed or, optionally, fed separately. Alternatively, the antiparasitic compounds may be administered to animals parenterally, for example, by intraruminal, intramuscular, intratracheal, or subcutaneous injection, in which event the active ingredient is dissolved or dispersed in a liquid carrier vehicle. For parenteral administration, the active material is suitably admixed with an acceptable vehicle, preferably of the vegetable oil variety, such as peanut oil, cotton seed oil and the like. Other parenteral vehicles, such as organic preparations using solketal, glycerol, formal and aqueous parenteral formulations, are also used. The active compound or compounds are dissolved or suspended in the parenteral formulation for administration; such formulations generally contain from 0.005 to 5% by weight of the active compound.

When the toxins are administered as a component of the feed of the animals, or dissolved or suspended in the drinking water, compositions are provided in which the active compound or compounds are intimately dispersed in an inert carrier or diluent. By inert carrier is meant one that will not react with the antiparasitic agent and one that may be administered safely to animals. Preferably, a carrier for feed administration is one that is, or may be, an ingredient of the animal ration.

Suitable compositions include feed premixes or supplements in which the active ingredient is present in relatively large amounts and which are suitable for direct feeding to the animal or for addition to the feed either directly or after an intermediate dilution or blending step. Typical carriers or diluents suitable for such compositions include, for example, distillers' dried grains, corn meal, citrus meal, fermentation residues, ground oyster shells, wheat shorts, molasses solubles, corn cob meal, edible bean mill feed, soya grits, crushed limestone and the like.

The toxin genes or gene fragments of the subject invention can be introduced into a wide variety of microbial hosts. Expression of the toxin gene results, directly or indirectly, in the intracellular production and maintenance of the nematicide. With suitable hosts, e.g., *Pseudomonas*, the microbes can be applied to the situs of nematodes where they will proliferate and be ingested by the nematodes. The result is a control of the nematodes. Alternatively, the microbe hosting the toxin gene can be treated under conditions that prolong the activity of the toxin produced in the cell. The treated cell then can be applied to the environment of target pest(s). The resulting product retains the toxicity of the *B.t.* toxin.

Where the *B.t.* toxin gene or gene fragment is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, it is essential that certain host microbes be used. Microorganism hosts are selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in

the particular environment (crop and other insect habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the nematicide from environmental degradation and inactivation.

5 A large number of microorganisms are known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots) of a wide variety of important crops. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., genera *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*,
10 *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylophilus*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*, *Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*; fungi, particularly yeast, e.g., genera *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium*. Of particular interest are such phytosphere bacterial species as *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Acetobacter xylinum*, *Agrobacterium tumefaciens*, *Rhodopseudomonas spheroides*, *Xanthomonas campestris*, *Rhizobium melioli*, *Alcaligenes entrophus*, and *Azotobacter vinlandii*; and phytosphere yeast species such as
15 *Rhodotorula rubra*, *R. glutinis*, *R. marina*, *R. aurantiaca*, *Cryptococcus albidus*, *C. diffluens*, *C. laurentii*, *Saccharomyces rosei*, *S. pretoriensis*, *S. cerevisiae*, *Sporobolomyces roseus*, *S. odoratus*, *Kluyveromyces veronae*, and *Aureobasidium pollulans*. Of particular interest are the pigmented microorganisms.

20 A wide variety of ways are known and available for introducing the *B.t.* genes or gene fragments expressing the toxin into the microorganism host under conditions which allow for stable maintenance and expression of the gene. The transformants can be isolated in accordance with conventional ways, usually employing a selection technique, which allows for selection of the desired organism as against unmodified organisms or transferring organisms, when present. The
25 transformants then can be tested for nematicidal activity.

Suitable host cells, where the nematicide-containing cells will be treated to prolong the activity of the toxin in the cell when the then treated cell is applied to the environment of target pest(s), may include either prokaryotes or eukaryotes, normally being limited to those cells which do not produce substances toxic to higher organisms, such as mammals. However, organisms
30 which produce substances toxic to higher organisms could be used, where the toxin is unstable or the level of application sufficiently low as to avoid any possibility of toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and the lower eukaryotes, such as fungi. Illustrative prokaryotes, both Gram-negative and -positive, include Enterobacteriaceae, such as *Escherichia*, *Erwinia*, *Shigella*, *Salmonella*, and *Proteus*; Bacillaceae; Rhizobiceae, such as *Rhizobium*; Spirillaceae, such as photobacterium, *Zymomonas*, *Serratia*, *Aeromonas*, *Vibrio*,
35 *Desulfovibrio*, *Spirillum*; Lactobacillaceae; Pseudomonadaceae, such as *Pseudomonas* and *Acetobacter*; Azotobacteraceae and Nitrobacteraceae. Among eukaryotes are fungi, such as Phycomycetes and Ascomycetes, which includes yeast, such as *Saccharomyces* and

Schizosaccharomyces; and Basidiomycetes yeast, such as *Rhodotorula*, *Aureobasidium*, *Sporobolomyces*, and the like.

Characteristics of particular interest in selecting a host cell for purposes of production include ease of introducing the *B.t.* gene or gene fragment into the host, availability of expression systems, efficiency of expression, stability of the nematicide in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a nematicide microcapsule include protective qualities for the nematicide, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; leaf affinity; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

Host organisms of particular interest include yeast, such as *Rhodotorula* sp., *Aureobasidium* sp., *Saccharomyces* sp., and *Sporobolomyces* sp.; phylloplane organisms such as *Pseudomonas* sp., *Erwinia* sp. and *Flavobacterium* sp.; or such other organisms as *Escherichia*, *Lactobacillus* sp., *Bacillus* sp., and the like. Specific organisms include *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae*, *Bacillus thuringiensis*, *Escherichia coli*, *Bacillus subtilis*, and the like.

The cell will usually be intact and be substantially in the proliferative form when treated, rather than in a spore form, although in some instances spores may be employed.

Treatment of the microbial cell, e.g., a microbe containing the *B.t.* toxin gene or gene fragment, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the toxin, nor diminish the cellular capability in protecting the toxin. Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable techniques include treatment with aldehydes, such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Bouin's fixative and Helly's fixative (See: Humason, Gretchen L., Animal Tissue Techniques, W.H. Freeman and Company, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the toxin produced in the cell when the cell is administered to the host animal. Examples of physical means are short wavelength radiation such as gamma-radiation and X-radiation, freezing, UV irradiation, lyophilization, and the like.

The cells generally will have enhanced structural stability which will enhance resistance to environmental conditions. Where the pesticide is in a proform, the method of inactivation should be selected so as not to inhibit processing of the proform to the mature form of the pesticide by the target pest pathogen. For example, formaldehyde will crosslink proteins and could inhibit processing of the proform of a polypeptide pesticide. The method of inactivation or killing retains at least a substantial portion of the bio-availability or bioactivity of the toxin.

The cellular host containing the *B.t.* nematocidal gene or gene fragment may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the *B.t.* gene or gene fragment. These cells may then be harvested in accordance with conventional ways. Alternatively, the cells can be treated prior to harvesting.

The various methods employed in the preparation of the plasmids and transformation of host organisms are well known in the art. These procedures are all described in Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York. Thus, it is within the skill of those in the genetic engineering art to extract DNA from microbial cells, perform restriction enzyme digestions, electrophorese DNA fragments, tail and anneal plasmid and insert DNA, ligate DNA, transform cells, prepare plasmid DNA, electrophorese proteins, and sequence DNA.

The *B.t.* cells may be formulated in a variety of ways. They may be employed as wettable powders, granules or dusts, by mixing with various inert materials, such as inorganic minerals (phyllosilicates, carbonates, sulfates, phosphates, and the like) or botanical materials (powdered corncobs, rice hulls, walnut shells, and the like). The formulations may include spreader-sticker adjuvants, stabilizing agents, other pesticidal additives, or surfactants. Liquid formulations may be aqueous-based or non-aqueous and employed as foams, gels, suspensions, emulsifiable concentrates, or the like. The ingredients may include rheological agents, surfactants, emulsifiers, dispersants, or polymers.

The nematocide concentration will vary widely depending upon the nature of the particular formulation, particularly whether it is a concentrate or to be used directly. The nematocide will be present in at least 1% by weight and may be 100% by weight. The dry formulations will have from about 1-95% by weight of the nematocide while the liquid formulations will generally be from about 1-60% by weight of the solids in the liquid phase. The formulations will generally have from about 10^2 to about 10^4 cells/mg. These formulations will be administered at about 50 mg (liquid or dry) to 1 kg or more per hectare.

The formulations can be applied to the environment of the nematodes, e.g., plants, soil or water, by spraying, dusting, sprinkling, or the like.

Following are examples which illustrate procedures, including the best mode, for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 – Culturing *B.t.* Isolates of the Invention

A subculture of a *B.t.* isolate can be used to inoculate the following medium, a peptone, glucose, salts medium.

Bacto Peptone	7.5 g/l
Glucose	1.0 g/l

19

	KH_2PO_4	3.4 g/l
	K_2HPO_4	4.35 g/l
	Salts Solution	5.0 ml/l
	CaCl_2 Solution	5.0 ml/l
5	Salts Solution (100 ml)	
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	2.46 g
	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.04 g
	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.28 g
	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.40 g
10	CaCl_2 Solution (100 ml)	
	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	3.66 g
	pH 7.2	

The salts solution and CaCl_2 solution are filter-sterilized and added to the autoclaved and cooked broth at the time of inoculation. Flasks are incubated at 30°C on a rotary shaker at 200 rpm for 64 hr.

Example 2 – Purification of Protein and Amino Acid Sequencing

The *B.t.* isolates PS17, PS63B, PS52A1, and PS69D1 were cultured as described in Example 1. The parasporal inclusion bodies were partially purified by sodium bromide (28-38%) isopycnic gradient centrifugation (Pfannenstiel, M.A., E.J. Ross, V.C. Kramer, and K.W. Nickerson [1984] FEMS Microbiol. Lett. 21:39). The proteins toxic for the nematode *Caenorhabditis elegans* were bound to PVDF membranes (Millipore, Bedford, MA) by western blotting techniques (Towbin, H., T. Staehlelin, and K. Gordon [1979] Proc. Natl. Acad. Sci. USA 76:4350) and the N-terminal amino acid sequences were determined by the standard Edman reaction with an automated gas-phase sequenator (Hunkapiller, M.W., R.M. Hewick, W.L. Dreyer, and L.E. Hood [1983] Meth. Enzymol. 91:399). The sequences obtained were:

PS17a: A I L N E L Y P S V P Y N V (SEQ ID NO. 17)
 PS17b: A I L N E L Y P S V P Y N V (SEQ ID NO. 18)
 PS52A1: M I I D S K T T L P R H S L I N T (SEQ ID NO. 19)
 PS63B: Q L Q A Q P L I P Y N V L A (SEQ ID NO. 20)
 PS69D1: M I L G N G K T L P K H I R L A H I F A T Q N S (SEQ ID NO. 21)
 PS33F2: A T L N E V Y P V N (SEQ ID NO. 22)

In addition, internal amino acid sequence data were derived for PS63B. The toxin protein was partially digested with *Staphylococcus aureus* V8 protease (Sigma Chem. Co., St. Louis, MO) essentially as described (Cleveland, D.W., S.G. Fischer, M.W. Kirschner, and U.K. Laemmli [1977] J. Biol. Chem. 252:1102). The digested material was blotted onto PVDF membrane and a ca. 28 kDa limit peptide was selected for N-terminal sequencing as described above. The sequence obtained was:

PS63B(2) V Q R I L D E K L S F Q L I K (SEQ ID NO. 23)

From these sequence data oligonucleotide probes were designed by utilizing a codon frequency table assembled from available sequence data of other *B.t.* toxin genes. The probes were synthesized on an Applied Biosystems, Inc. DNA synthesis machine.

Protein purification and subsequent amino acid analysis of the N-terminal peptides listed above has led to the deduction of several oligonucleotide probes for the isolation of toxin genes from nematocidal *B.t.* isolates. RFLP analysis of restricted total cellular DNA using radiolabeled oligonucleotide probes has elucidated different genes or gene fragments.

Example 3 - Cloning of Novel Toxin Genes and Transformation into *Escherichia coli*

Total cellular DNA was prepared by growing the cells *B.t.* PS17 to a low optical density ($OD_{600} = 1.0$) and recovering the cells by centrifugation. The cells were protoplasted in TES buffer (30 mM Tris-Cl, 10 mM EDTA, 50 mM NaCl, pH = 8.0) containing 20 % sucrose and 50 mg/ml lysozyme. The protoplasts were lysed by addition of SDS to a final concentration of 4%. The cellular material was precipitated overnight at 4°C in 100 mM (final concentration) neutral potassium chloride. The supernate was extracted twice with phenol/chloroform (1:1). The DNA was precipitated with ethanol and purified by isopycnic banding on a cesium chloride-ethidium bromide gradient.

Total cellular DNA from PS17 was digested with *EcoRI* and separated by electrophoresis on a 0.8% (w/v) Agarose-TAE (50 mM Tris-HCl, 20 mM NaOAc, 2.5 mM EDTA, pH=8.0) buffered gel. A Southern blot of the gel was hybridized with a [^{32}P] - radiolabeled oligonucleotide probe derived from the N-terminal amino acid sequence of purified 130 kDa protein from PS17. The sequence of the oligonucleotide synthesized is (GCAATTTTAAATGAATTATATCC) (SEQ ID NO. 24). Results showed that the hybridizing *EcoRI* fragments of PS17 are 5.0 kb, 4.5 kb, 2.7 kb and 1.8 kb in size, presumptively identifying at least four new nematode-active toxin genes, PS17d, PS17b, PS17a and PS17e, respectively.

A library was constructed from PS17 total cellular DNA partially digested with *Sau3A* and size fractionated by electrophoresis. The 9 to 23 kb region of the gel was excised and the DNA was electroeluted and then concentrated using an ElutipTM ion exchange column (Schleicher and Schuel, Keene NH). The isolated *Sau3A* fragments were ligated into LambdaGEM-11TM (PROMEGA). The packaged phage were plated on KW251 *E. coli* cells (PROMEGA) at a high titer and screened using the above radiolabeled synthetic oligonucleotide as a nucleic acid hybridization probe. Hybridizing plaques were purified and rescreened at a lower plaque density. Single isolated purified plaques that hybridized with the probe were used to infect KW251 *E. coli* cells in liquid culture for preparation of phage for DNA isolation. DNA was isolated by standard procedures.

Recovered recombinant phage DNA was digested with *EcoRI* and separated by electrophoresis on a 0.8% agarose-TAE gel. The gel was Southern blotted and hybridized with the oligonucleotide probe to characterize the toxin genes isolated from the lambda library. Two

patterns were present, clones containing the 4.5 kb (PS17b) or the 2.7 kb (PS17a) *EcoRI* fragments. Preparative amounts of phage DNA were digested with *SaII* (to release the inserted DNA from lambda arms) and separated by electrophoresis on a 0.6% agarose-TAE gel. The large fragments, electroeluted and concentrated as described above, were ligated to *SaII*-digested and dephosphorylated pBCLac, an *E. coli/B.t.* shuttle vector comprised of replication origins from pBC16 and pUC19. The ligation mix was introduced by transformation into NM522 competent *E. coli* cells and plated on LB agar containing ampicillin, isopropyl-(Beta)-D-thiogalactoside (IPTG) and 5-Bromo-4-Chloro-3-indolyl-(Beta)-D-galactoside (XGAL). White colonies, with putative insertions in the (Beta)-galactosidase gene of pBCLac, were subjected to standard rapid plasmid purification procedures to isolate the desired plasmids. The selected plasmid containing the 2.7 kb *EcoRI* fragment was named pMYC1627 and the plasmid containing the 4.5 kb *EcoRI* fragment was called pMYC1628.

The toxin genes were sequenced by the standard Sanger dideoxy chain termination method using the synthetic oligonucleotide probe, disclosed above, and by "walking" with primers made to the sequence of the new toxin genes.

The PS17 toxin genes were subcloned into the shuttle vector pHT3101 (Lereclus, D. et al. [1989] FEMS Microbiol. Lett. 60:211-218) using standard methods for expression in *B.t.* Briefly, *SaII* fragments containing the 17a and 17b toxin genes were isolated from pMYC1629 and pMYC1627, respectively, by preparative agarose gel electrophoresis, electroelution, and concentrated, as described above. These concentrated fragments were ligated into *SaII*-cleaved and dephosphorylated pHT3101. The ligation mixtures were used separately to transform frozen, competent *E. coli* NM522. Plasmids from each respective recombinant *E. coli* strain were prepared by alkaline lysis and analyzed by agarose gel electrophoresis. The resulting subclones, pMYC2311 and pMYC2309, harbored the 17a and 17b toxin genes, respectively. These plasmids were transformed into the acrySTALLIFEROUS *B.t.* strain, HD-1 *cryB* (Aronson, A., Purdue University, West Lafayette, IN), by standard electroporation techniques (Instruction Manual, Biorad, Richmond, CA).

Recombinant *B.t.* strains HD-1 *cryB* [pMYC2311] and [pMYC2309] were grown to sporulation and the proteins purified by NaBr gradient centrifugation as described above for the wild-type *B.t.* proteins.

Example 4 – Activity of the *B.t.* Toxin Protein and Gene Product Against *Caenorhabditis elegans*

Caenorhabditis elegans (CE) was cultured as described by Simpkin and Coles (J. Chem. Tech. Biotechnol. 31:66-69, 1981) in corning (Corning Glass Works, Corning, NY) 24-well tissue culture plates containing 1 ml S-basal media, 0.5 mg ampicillin and 0.01 mg cholesterol. Each well also contained *ca.* 10^8 cells of *Escherichia coli* strain OP-50, a uracil auxotroph. The wells were seeded with *ca.* 100-200 CE per well and incubated at 20°C. Samples of protein (obtained from the wild type *B.t.* or the recombinant *B.t.*) were added to the wells by serial dilution. Water served as the control as well as the vehicle to introduce the proteins to the wells.

Each of the wells were examined daily and representative results are as follows:

μ g Toxin	% Kill with protein from indicated isolate		
	HD-1 cryB [pMYC2309]	HD-1 cryB [pMYC 2311]	PS17
100	25	50	75
32	25	50	75
10	50	25	50
1	0	0	0

Example 5 – Molecular Cloning of Gene Encoding a Novel Toxin From *Bacillus thuringiensis* strain PS52A1

Total cellular DNA was prepared from *Bacillus thuringiensis* PS52A1 (B.t. PS52A1) as disclosed in Example 3.

RFLP analyses were performed by standard hybridization of Southern blots of PS52A1 DNA with a 32 P-labeled oligonucleotide probe designed from the N-terminal amino acid sequence disclosed in Example 2. The sequence of this probe is:

5' ATG ATT ATT GAT TCT AAA ACA ACA TTA CCA AGA CAT TCA/T
TTA ATA/T AAT ACA/T ATA/T AA 3' (SEQ ID NO. 25)

This probe was designated 52A1-C. Hybridizing bands included an approximately 3.6 kbp *Hind*III fragment and an approximately 8.6 kbp *Eco*RV fragment. A gene library was constructed from PS52A1 DNA partially digested with *Sau*3A. Partial restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 6.6 to 23 kbp in size were excised from the gel, electroeluted from the gel slice, and recovered by ethanol precipitation after purification on an Elutip-D ion exchange column. The *Sau*3A inserts were ligated into *Bam*HI-digested LambdaGem-11 (Promega). Recombinant phage were packaged and plated on *E. coli* KW251 cells (Promega). Plaques were screened by hybridization with the radiolabeled 52A1-C oligonucleotide probe disclosed above. Hybridizing phage were plaque-purified and used to infect liquid cultures of *E. coli* KW251 cells for isolation of phage DNA by standard procedures (Maniatis et al.). For subcloning, preparative amounts of DNA were digested with *Eco*RI and *Sal*I, and electrophoresed on an agarose gel. The approximately 3.1 kbp band containing the toxin gene was excised from the gel, electroeluted from the gel slice, and purified by ion exchange chromatography as above. The purified DNA insert was ligated into *Eco*RI + *Sal*I-digested pHTBlueII (an *E. coli*/*B. thuringiensis* shuttle vector comprised of pBluescript S/K [Stratagene] and the replication origin from a resident *B.t.* plasmid [D. Lereclus et al. 1989. FEMS Microbiology Letters 60:211-218]). The ligation mix was used to transform frozen, competent *E. coli* NM522 cells (ATCC 47000). Transformants were plated on LB agar containing ampicillin, isopropyl-(Beta)-D-thiogalactoside (IPTG), and 5-Bromo-4-Chloro-3-indolyl-(Beta)-D-galactoside (XGAL).

Plasmids were purified from putative recombinants by alkaline lysis (Maniatis et al.) and analyzed by electrophoresis of *Eco*RI and *Sal*II digests on agarose gels. The desired plasmid construct, pMYC2321 contains a toxin gene that is novel compared to the maps of other toxin genes encoding nematocidal proteins.

5 Plasmid pMYC2321 was introduced into an acrySTALLIFEROUS (Cry^-) *B.t.* host by electroporation. Expression of an approximately 55-60 kDa crystal protein was verified by SDS-PAGE analysis. NaBr-purified crystals were prepared as described in Example 3 for determination of toxicity of the cloned gene product to *Pratylenchus* spp.

10 Example 6 – Activity of the *B.t.* PS52A1 Toxin Protein and Gene Product Against the Root Lesion Nematode, *Pratylenchus scribneri*

Pratylenchus scribneri was reared aseptically on excised corn roots in Gamborg's B5 medium (GIBCO Laboratories, Grand Island, NY). Bioassays were done in 24 well assay plates (Corning #25820) using L 3-4 larvae as described by Tsai and Van Gundy (J. Nematol. 22(3):327-15 332). Approximately 20 nematodes were placed in each well. A total of 80-160 nematodes were used in each treatment. Samples of protein were suspended in aqueous solution using a hand-held homogenizer.

Mortality was assessed by prodding with a dull probe 7 days after treatment. Larvae that did not respond to prodding were considered moribund. Representative results are shown below.

	Rate (ppm)	Percent Moribund
20	200	75
25	Control	5

Example 7 – Molecular Cloning of Gene Encoding a Novel Toxin From *Bacillus Thuringiensis* strain PS69D1

Total cellular DNA was prepared from PS69D1 (*B.t.* PS69D1) as disclosed in Example 3. RFLP analyses were performed by standard hybridization of Southern blots of PS69D1 DNA with a ^{32}P -labeled oligonucleotide probe designated as 69D1-D. The sequence of the 69D1-D probe was:

5' AAA CAT ATT AGA TTA GCA CAT ATT TTT GCA ACA CAA AA 3'
(SEQ ID NO. 26)

35 Hybridizing bands included an approximately 2.0 kbp *Hind*III fragment.

A gene library was constructed from PS69D1 DNA partially digested with *Sau*3A. Partial restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 6.6 to 23 kbp in size were excised from the gel, electroeluted from the gel slice, and recovered by ethanol precipitation after purification on an Elutip-D ion exchange column. The *Sau*3A inserts were ligated into *Bam*HI-digested LambdaGem-11 (Promega, Madison, WI). Recombinant phage were packaged and plated on *E. coli* KW251 cells (Promega, Madison, WI). Plaques were screened by

hybridization with the radiolabeled 69D1-D oligonucleotide probe. Hybridizing phage were plaque-purified and used to infect liquid cultures of *E. coli* KW251 cells for isolation of phage DNA by standard procedures (Maniatis et al. [1982] *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY). For subcloning, preparative amounts of DNA were digested with *Hind*III and electrophoresed on an agarose gel. The approximately, 2.0 kbp band containing the toxin gene was excised from the gel, electroeluted from the gel slice, and purified by ion exchange chromatography as above. The purified DNA insert was ligated into *Hind*III-digested pHTBlueII (and *E. coli*/B.t. shuttle vector comprised of pBluescript S/K (Stratagene, San Diego, CA) and the replication origin from a resident B.t. plasmid (D. Lereclus et al [1989] FEMS Microbiol. Lett. 60:211-218). The ligation mix was used to transform frozen, competent *E. coli* NM522 cells (ATCC 47000). Transformants were plated on LB agar containing 5-bromo-4-chloro-3-indolyl- (Beta)-D-galactoside (XGAL). Plasmids were purified from putative recombinants by alkaline lysis (Maniatis et al., *supra*) and analyzed by electrophoresis of *Hind*III digests on agarose gels. The desired plasmid construct, pMYC2317, contains a toxin gene that is novel compared to the maps of other toxin genes encoding insecticidal proteins.

Example 8 – Molecular Cloning of a Gene Encoding a Novel Toxin from *Bacillus thuringiensis* Strain PS63B

Example 2 shows the aminoterminal and internal polypeptide sequences of the PS63B toxin protein as determined by standard Edman protein sequencing. From these sequences, two oligonucleotide primers were designed using a codon frequency table assembled from B.t. genes encoding δ -endotoxins. The sequence of the forward primer (63B-A) was complementary to the predicted DNA sequence at the 5' end of the gene:

63B-A - 5' CAA T/CTA CAA GCA/T CAA CC 3' (SEQ ID NO. 27)

The sequence of the reverse primer (63B-INT) was complementary to the inverse of the internal predicted DNA sequence:

63B-INT - 5' TTC ATC TAA AAT TCT TTG A/TAC 3' (SEQ ID NO. 28)

These primers were used in standard polymerase chain reactions (Cetus Corporation) to amplify an approximately 460 bp fragment of the 63B toxin gene for use as a DNA cloning probe. Standard Southern blots of total cellular DNA from PS63B were hybridized with the radiolabeled PCR probe. Hybridizing bands included an approximately 4.4 kbp *Xba*I fragment, an approximately 2.0 kbp *Hind*III fragment, and an approximately 6.4 kbp *Spe*I fragment.

Total cellular DNA was prepared from *Bacillus thuringiensis* (B.t.) cells grown to an optical density of 1.0 at 600 nm. The cells were recovered by centrifugation and protoplasts were prepared in lysis mix (300 mM sucrose, 25 mM Tris-HCl, 25 mM EDTA, pH = 8.0) and lysozyme at a concentration of 20 mg/ml. The protoplasts were ruptured by addition of ten volumes of 0.1 M NaCl, 0.1 M Tris-HCl pH 8.0, and 0.1% SDS. The cellular material was quickly frozen at -70°C and thawed to 37°C twice. The supernatant was extracted twice with phenol/chloroform (1:1). The nucleic acids were precipitated with ethanol. To remove as much RNA as possible

from the DNA preparation, RNase at final concentration of 200 μ g/ml was added. After incubation at 37°C for 1 hour, the solution was extracted once with phenol/chloroform and precipitated with ethanol.

5 A gene library was constructed from PS63B total cellular DNA partially digested with *Nde*II and size fractionated by gel electrophoresis. The 9-23 kb region of the gel was excised and the DNA was electroeluted and then concentrated using an Elutip-d ion exchange column (Schleicher and Schuel, Keene, NH). The isolated *Nde*II fragments were ligated into *Bam*HI-digested LambdaGEM-11 (PROMEGA). The packaged phage were plated on *E. coli* KW251 cells (PROMEGA) at a high titer and screened using the radiolabeled approximately 430 bp fragment
10 probe amplified with the 63B-A and 63B internal primers (SEQ ID NOS. 27 and 28, respectively) by polymerase chain reaction. Hybridizing plaques were purified and rescreened at a lower plaque density. Single isolated, purified plaques that hybridized with the probe were used to infect KW251 cells in liquid culture for preparation of phage for DNA isolation. DNA was isolated by standard procedures (Maniatis et al., *supra*). Preparative amounts of DNA were digested with *Sal*I
15 (to release the inserted DNA from lambda sequences) and separated by electrophoresis on a 0.6% agarose-TAE gel. The large fragments were purified by ion exchange chromatography as above and ligated to *Sal*I-digested, dephosphorylated pHTBlueII (an *E. coli*/*B.t.* shuttle vector comprised of pBlueScript S/K [Stratagene, San Diego, CA] and the replication origin from a resident *B.t.* plasmid [Lereclus, D. et al. (1989) FEMS Microbiol. Lett. 60:211-218]). The ligation mix was
20 introduced by transformation into competent *E. coli* NM522 cells (ATCC 47000) and plated on LB agar containing ampicillin (100 μ g/ml), IPTG (2%), and XGAL (2%). White colonies, with putative restriction fragment insertions in the (Beta)-galactosidase gene of pHTBlueII, were subjected to standard rapid plasmid purification procedures (Maniatis et al., *supra*). Plasmids were
25 analyzed by *Sal*I digestion and agarose gel electrophoresis. The desired plasmid construct, pMYC1641, contains an approximately 14 kb *Sal*I insert.

For subcloning, preparative amounts of DNA were digested with *Xba*I and electrophoresed on an agarose gel. The approximately 4.4 kbp band containing the toxin gene was excised from the gel, electroeluted from the gel slice, and purified by ion exchange chromatography as above. This fragment was ligated into *Xba*I cut pHTBlueII and the resultant
30 plasmid was designated pMYC1642.

Example 9 - Cloning of a Novel Toxin Gene From *B.t.* PS33F2 and Transformation into *Escherichia coli*

35 Total cellular DNA was prepared from *B.t.* PS33F2 cells grown to an optical density, at 600 nm, of 1.0. Cells were pelleted by centrifugation and resuspended in protoplast buffer (20 mg/ml lysozyme in 0.3 M sucrose, 25 mM Tris-Cl [pH 8.0], 25 mM EDTA). After incubation at 37°C for 1 hour, protoplasts were lysed by the addition of nine volumes of a solution of 0.1 M NaCl, 0.1% SDS, 0.1 M Tris-Cl followed by two cycles of freezing and thawing. The cleared lysate was extracted twice with phenol:chloroform (1:1). Nucleic acids were precipitated with two

volumes of ethanol and pelleted by centrifugation. The pellet was resuspended in 10 mM Tris-Cl, 1 mM EDTA (TE) and RNase was added to a final concentration of 50 µg/ml. After incubation at 37°C for 1 hour, the solution was extracted once each with phenol:chloroform (1:1) and TE-saturated chloroform. DNA was precipitated from the aqueous phase by the addition of one-tenth
5 volume of 3 M NaOAc and two volumes of ethanol. DNA was pelleted by centrifugation, washed with 70% ethanol, dried, and resuspended in TE.

Plasmid DNA was extracted from protoplasts prepared as described above. Protoplasts were lysed by the addition of nine volumes of a solution of 10 mM Tris-Cl, 1 mM EDTA, 0.085 N NaOH, 0.1% SDS, pH=8.0. SDS was added to 1% final concentration to complete lysis. One-
10 half volume of 3 M KOAc was then added and the cellular material was precipitated overnight at 4°C. After centrifugation, the DNA was precipitated with ethanol and plasmids were purified by isopycnic centrifugation on cesium chloride-ethidium bromide gradients.

Restriction Fragment Length Polymorphism (RFLP) analyses were performed by standard hybridization of Southern blots of PS33F2 plasmid and total cellular DNA with ³²P-labelled
15 oligonucleotide probes designed to the N-terminal amino acid sequence disclosed in Example 2.

Probe 33F2A: 5' GCA/T ACA/T TTA AAT GAA GTA/T TAT 3' (SEQ ID NO. 33)

Probe 33F2B: 5' AAT GAA GTA/T TAT CCA/T GTA/T AAT 3' (SEQ ID NO. 34)

Hybridizing bands included an approximately 5.85 kbp *Eco*RI fragment. Probe 33F2A and a reverse PCR primer were used to amplify a DNA fragment of approximately 1.8 kbp for use as
20 a hybridization probe for cloning the PS33F2 toxin gene. The sequence of the reverse primer was:
5' GCAAGCGGCCGCTTATGGAATAAATTCAATT C/T T/G A/G TC T/A A 3'
(SEQ ID NO. 35).

A gene library was constructed from PS33F2 plasmid DNA digested with *Eco*RI. Restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 4.3-6.6 kbp
25 were excised from the gel, electroeluted from the gel slice, and recovered by ethanol precipitation after purification on an Elutip-D ion exchange column (Schleicher and Schuel, Keene NH). The *Eco*RI inserts were ligated into *Eco*RI-digested pHTBlueII (an *E. coli*/*B. thuringiensis* shuttle vector comprised of pBluescript S/K [Stratagene] and the replication origin from a resident *B.t.* plasmid [D. Lereclus et al. 1989. FEMS Microbial. Lett. 60:211-218]). The ligation mixture was
30 transformed into frozen, competent NM522 cells (ATCC 47000). Transformants were plated on LB agar containing ampicillin, isopropyl -(Beta)-D-thiogalactoside (IPTG), and 5-bromo-4-chloro-3-indolyl-(Beta)-D-galactoside (XGAL). Colonies were screened by hybridization with the radiolabeled PCR amplified probe described above. Plasmids were purified from putative toxin gene clones by alkaline lysis and analyzed by agarose gel electrophoresis of restriction digests. The
35 desired plasmid construct, pMYC2316, contains an approximately 5.85 kbp *Eco*RI insert; the toxin gene residing on this DNA fragment (33F2a) is novel compared to the DNA sequences of other toxin genes encoding nematocidal proteins.

Plasmid pMYC2316 was introduced into the acrySTALLIFEROUS (Cry-) *B.t.* host, HD-1 CryB (A. Aronson, Purdue University, West Lafayette, IN) by electroporation. Expression of an

approximately 120-140 kDa crystal protein was verified by SDS-PAGE analysis. Crystals were purified on NaBr gradients (M.A. Pfannenstiel et al. 1984. FEMS Microbiol. Lett. 21:39) for determination of toxicity of the cloned gene product to *Pratylenchus* spp.

5 Example 10 – Activity of the *B.t.* Gene Product 33F2 Against the Plant Nematode *Pratylenchus* spp.

Pratylenchus spp. was reared aseptically on excised corn roots in Gamborg's B5 medium (GIBCO® Laboratories, Grand Island, NY) Bioassays were done in 24 well assay plates (Corning #25820) using L 3-4 larvae as described by Tsai and van Gundy (J. Nematol. 22(3):327-332).
10 Approximately 20 nematodes were placed in each well. A total of 80-160 nematodes were used in each treatment. Samples of protein were suspended in an aqueous solution using a hand-held Dounce homogenizer.

Mortality was assessed visually 3 days after treatment. Larvae that were nearly straight and not moving were considered moribund. Representative results are as follows:

33F2a (ppm)	% Moribund
0	12
75	78

Species of *Pratylenchus*, for example *P. scribneri*, are known pathogens of many economically important crops including corn, peanuts, soybean, alfalfa, beans, tomato, and citrus. These "root lesion" nematodes are the second most economically damaging genus of plant parasitic nematodes (after *Meloidogyne*—the "root knot" nematode), and typify the migratory endoparasites.
25

Example 11 – Cloning of Novel Nematode-Active Genes Using Generic Oligonucleotide Primers

The nematocidal gene of a new nematocidal *B.t.* can be obtained from DNA of the strain by performing the standard polymerase chain reaction procedure as in Example 8 using the oligonucleotides of SEQ ID NO. 32 or SEQ ID NO. 30 as reverse primers and SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 24, Probe B of SEQ ID NO. 5 (AAT GAA GTA/T TAT CCA/T GTA/T AAT), or SEQ ID NO. 27 as forward primers. The expected PCR fragments would be approximately 330 to 600 bp (with either reverse primer and SEQ ID NO. 14), 1000 to 1400 bp (with either reverse primer and SEQ ID NO. 16), and 1800 to 2100 bp (with either reverse primer and any of the three N-terminal primers, SEQ ID NO. 5 (Probe B), SEQ ID NO. 24, and SEQ ID NO. 27). Alternatively, a complement from the primer family described by SEQ ID NO. 14 can be used as reverse primer with SEQ ID NO. 16, SEQ ID NO. 24, SEQ ID NO. 5 (Probe B), or SEQ ID NO. 27 as forward primers. The expected PCR fragments would be approximately 650 to 1000 bp with SEQ ID NO. 16, and 1400 to 1800 bp (for the three N-terminal primers, SEQ ID NO. 5 (Probe B), SEQ ID NO. 24, and SEQ ID NO. 27). Amplified DNA fragments of the indicated sizes can be radiolabeled and used as probes to clone the entire gene as in Example 8.
40

Example 12 – Further Cloning of Novel Nematode-Active Genes Using Generic Oligonucleotide Primers

A gene coding for a nematocidal toxin a new nematocidal *B.t.* isolate can also be obtained from DNA of the strain by performing the standard polymerase chain reaction procedure as in

5 Example 8 using oligonucleotides derived from the PS52A1 and PS69D1 gene sequences as follows:

1. Forward primer "TGATTTT(T or A)(C or A)TCAATTATAT(A or G)A(G or T)GTTTAT" (SEQ ID NO. 36) can be used with primers complementary to probe "AAGAGTTA(C or T)TA(A or G)A(G or A)AAAGTA" (SEQ ID NO. 37), probe

10 "TTAGGACCATT(A or G)(C or T)T(T or A)GGATTTGTTGT(A or T)TATGAAAT" (SEQ ID NO. 38), and probe "GA(C or T)AGAGATGT(A or T)AAAAT(C or T)(T or A)TAGGAATG" (SEQ ID NO. 39) to produce amplified fragments of approximately 440, 540, and 650 bp, respectively.

15 2. Forward primer "TT(A or C)TAAA(A or T)C(A or T)GCTAATGATATT" (SEQ ID NO. 40) can be used with primers complementary to SEQ ID NO. 37, SEQ ID NO. 38, and SEQ ID NO. 39 to produce amplified fragments of approximately 360, 460, and 570 bp, respectively.

20 3. Forward primer SEQ ID NO. 37 can be used with primers complementary to SEQ ID NO. 38 and SEQ ID NO. 39 to produce amplified fragments of approximately 100 and 215 bp, respectively.

Amplified DNA fragments of the indicated sizes can be radiolabeled and used as probes to clone the entire gene as in Example 8.

Example 13 – Insertion of Toxin Gene Into Plants

25 One aspect of the subject invention is the transformation of plants with genes coding for a nematocidal toxin. The transformed plants are resistant to attack by nematodes.

Genes coding for nematocidal toxins, as disclosed herein, can be inserted into plant cells using a variety of techniques which are well known in the art. For example, a large number of cloning vectors comprising a replication system in *E. coli* and a marker that permits selection of

30 the transformed cells are available for preparation for the insertion of foreign genes into higher plants. The vectors comprise, for example, pBR322, pUC series, M13mp series, pACYC184, etc. Accordingly, the sequence coding for the *B.t.* toxin can be inserted into the vector at a suitable restriction site. The resulting plasmid is used for transformation into *E. coli*. The *E. coli* cells are cultivated in a suitable nutrient medium, then harvested and lysed. The plasmid is recovered.

35 Sequence analysis, restriction analysis, electrophoresis, and other biochemical-molecular biological methods are generally carried out as methods of analysis. After each manipulation, the DNA sequence used can be cleaved and joined to the next DNA sequence. Each plasmid sequence can be cloned in the same or other plasmids. Depending on the method of inserting desired genes into the plant, other DNA sequences may be necessary. If, for example, the Ti or Ri plasmid is

used for the transformation of the plant cell, then at least the right border, but often the right and the left border of the Ti or Ri plasmid T-DNA, has to be joined as the flanking region of the genes to be inserted.

5 The use of T-DNA for the transformation of plant cells has been intensively researched and sufficiently described in EP 120 516; Hoekema (1985) In: *The Binary Plant Vector System*, Offset-durkkerij Kanters B.V., Alblasterdam, Chapter 5; Fraley *et al.*, Crit. Rev. Plant Sci. 4:1-46; and An *et al.* (1985) EMBO J. 4:277-287.

10 Once the inserted DNA has been integrated in the genome, it is relatively stable there and, as a rule, does not come out again. It normally contains a selection marker that confers on the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G 418, bleomycin, hygromycin, or chloramphenicol, *inter alia*. The individually employed marker should accordingly permit the selection of transformed cells rather than cells that do not contain the inserted DNA.

15 A large number of techniques are available for inserting DNA into a plant host cell. Those techniques include transformation with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as transformation agent, fusion, injection, or electroporation as well as other possible methods. If agrobacteria are used for the transformation, the DNA to be inserted has to be cloned into special plasmids, namely either into an intermediate vector or into a binary vector. The intermediate vectors can be integrated into the Ti or Ri plasmid by homologous recombination owing to sequences that are homologous to sequences in the T-DNA. The Ti or Ri plasmid also comprises the vir region necessary for the transfer of the T-DNA. Intermediate vectors cannot replicate themselves in agrobacteria. The intermediate vector can be transferred into *Agrobacterium tumefaciens* by means of a helper plasmid (conjugation). Binary vectors can replicate themselves both in *E. coli* and in agrobacteria. They comprise a selection marker gene and a linker or polylinker which are framed by the right and left T-DNA border regions. They can be transformed directly into agrobacteria (Holsters *et al.* [1978] Mol. Gen. Genet. 163:181-187). The agrobacterium used as host cell is to comprise a plasmid carrying a vir region. The vir region is necessary for the transfer of the T-DNA into the plant cell. Additional T-DNA may be contained. The bacterium so transformed is used for the transformation of plant cells. Plant explants can advantageously be cultivated with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* for the transfer of the DNA into the plant cell. Whole plants can then be regenerated from the infected plant material (for example, pieces of leaf, segments of stalk, roots, but also protoplasts or suspension-cultivated cells) in a suitable medium, which may contain antibiotics or biocides for selection. The plants so obtained can then be tested for the presence of the inserted DNA. No special demands are made of the plasmids in the case of injection and electroporation. It is possible to use ordinary plasmids, such as, for example, pUC derivatives.

20
25
30
35

The transformed cells grow inside the plants in the usual manner. They can form germ cells and transmit the transformed trait(s) to progeny plants. Such plants can be grown in the normal manner and crossed with plants that have the same transformed hereditary factors or other hereditary factors. The resulting hybrid individuals have the corresponding phenotypic properties.

5

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

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page 14BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO
Dr. Jewel Payne
Entomology
Mycogen Corporation
5457 Oberlin Dr.
San Diego, CA 92121
NAME AND ADDRESS
OF DEPOSITOR

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <u>Bacillus thuringiensis</u> PS17	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NRRL B-18243
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by:	
<input type="checkbox"/> a scientific description	
<input checked="" type="checkbox"/> a proposed taxonomic designation	
(Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on July 28, 1987 (date of the original deposit) ¹	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Agricultural Research Culture Collection (NRRL) International Depositary Authority Address: 1815 N. University Street Peoria, Illinois 61604 U.S.A.	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: <i>Albert J. Zorn</i> August 10, 1987

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired; where a deposit made outside the Budapest Treaty after the acquisition of the status of international depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authority.

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Identification reference given by the DEPOSITOR: - <u>Bacillus thuringiensis</u> PS33F2	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NRRL B-18244
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
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Identification reference given by the DEPOSITOR: - <u>Bacillus thuringiensis</u> PS 52A1	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NRRL B-18245
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
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Identification reference given by the DEPOSITOR: <u>Bacillus thuringiensis</u> PS63B	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NRRL B-18246
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by:	
<input type="checkbox"/> a scientific description <input checked="" type="checkbox"/> a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on July 28, 1987 (date of the original deposit) ¹	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Agricultural Research Culture Collection (NRRL) International Depositary Authority Address: 1815 N. University Street Peoria, Illinois 61604 U.S.A.	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: <i>Alfred J. Zorn</i> <i>August 10, 1987</i>

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired; where a deposit made outside the Budapest Treaty after the acquisition of the status of international depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authority.

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BP/A/II/12
page 14BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO

Dr. Jewel Payne
Entomology
Mycogen Corporation
5457 Oberlin Dr.
San Diego, CA 92121
NAME AND ADDRESS
OF DEPOSITORRECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: - <u>Bacillus thuringiensis</u> PS 69D1	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NRRL B-18247
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: <input type="checkbox"/> a scientific description <input checked="" type="checkbox"/> a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on July 28, 1987 (date of the original deposit) ¹	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Agricultural Research Culture Collection (NRRL) International Depositary Authority Address: 1815 N. University Street Peoria, Illinois 61604 U.S.A.	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: <i>Albert J. Zorn</i> August 10, 1987

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired; where a deposit made outside the Budapest Treaty after the acquisition of the status of international depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authority.

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICRO-ORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Ms. Lenore Linda R. Nygaard
Mycogen Corporation
5451 Oberlin Dr.
San Diego, CA 92121

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS
OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <u>Escherichia coli</u> NMS22/pMYC2316 MR608	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NRRL B-18785
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by:	
<input type="checkbox"/> a scientific description <input checked="" type="checkbox"/> a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on <u>Mar. 15, 1991</u> (date of the original deposit) ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on _____ (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on _____ (date of receipt of request for conversion)	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Agricultural Research Culture Collection (NRRL) International Depositary Authority Address: 1815 N. University Street Peoria, Illinois 61604 U.S.A.	Signature(s) of person(s) having the power to represent the International Depositary Authority, or/authorized official(s): Date: <u>March 27, 1991</u>

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT LAW

INTERNATIONAL FORM

Ms. Lenore Linda R. Nygaard
Mycogen Corporation
5451 Oberlin Dr.
San Diego, CA 92121

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS
OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <u>Escherichia coli</u> NM522/pMYC 2321 MR607	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NRRL B-18770
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by:	
<input type="checkbox"/> a scientific description	
<input checked="" type="checkbox"/> a proposed taxonomic designation	
(Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on Feb.14,1991 (date of the original deposit) ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Agricultural Research Culture Collection (NRRL) International Depositary Authority Address: 1815 N. University Street Peoria, Illinois 61604 U.S.A.	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: <i>Albert J. Zorn</i> February 26, 1991

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Ms. Lenore Linda R. Nygaard
Mycogen Corporation
5451 Oberlin Dr.
San Diego, CA 92121

NAME AND ADDRESS
OF DEPOSITOR

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the
DEPOSITOR:

Escherichia coli
NMS22/pMYC2317 MR609

Accession number given by the
INTERNATIONAL DEPOSITARY AUTHORITY:

NRRL B-18816

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☒ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above,
which was received by it on Apr. 24, 1991 (date of the original deposit);

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International
Depositary Authority on (date of the original deposit) and
a request to convert the original deposit to a deposit under the Budapest Treaty
was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: Agricultural Research Culture
Collection (NRRL)

International Depositary Authority

Address: 1815 N. University Street
Peoria, Illinois 61604 U.S.A.

Signature(s) of person(s) having the power
to represent the International Depositary
Authority or of authorized official(s):

Date:

James H. George

5-10-91

Where Rule 6.4(d) applies, such date is the date on which the status of international depositary
authority was acquired.

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page 14BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Ms. ^{TO} Lenore Linda R. Nygaard
Mycogen Corporation
5451 Oberlin Dr.
San Diego, CA 92121

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page.

NAME AND ADDRESS
OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <u>Escherichia coli</u> NM522/pMYC1627 MR398	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NRRL B-18651
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: <input type="checkbox"/> a scientific description <input checked="" type="checkbox"/> a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on May 11, 1990 (date of the original deposit) ¹	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Agricultural Research Culture Collection (NRRL) International Depositary Authority Address: 1815 N. University Street Peoria, Illinois 61604 U.S.A.	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: <i>Alfred J. Burn</i> May 17, 1990

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired; where a deposit made outside the Budapest Treaty after the acquisition of the status of international depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authority.

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page 14BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO
Ms. Lenore Linda R. Nygaard
Mycogen Corporation
5451 Oberlin Dr.
San Diego, CA 92121

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page.

NAME AND ADDRESS
OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <u>Escherichia coli</u> NM522/pMYC1623 MR399	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NRRL B-18652
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by:	
<input type="checkbox"/> a scientific description	
<input checked="" type="checkbox"/> a proposed taxonomic designation	
(Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on May 11, 1990 (date of the original deposit).	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Agricultural Research Culture Collection (NRRL) International Depositary Authority Address: 1815 N. University Street Peoria, Illinois 61604 U.S.A.	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: <u>Allen J. Zimm</u> May 17, 1990

Where Rule 6.4(d) applies, such date is the date on which the status of international depositary
authority was acquired; where a deposit made outside the Budapest Treaty after the acquisition
of the status of international depositary authority is converted into a deposit under the
Budapest Treaty, such date is the date on which the microorganism was received by the
international depositary authority.

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO
Ms. Lenore Linda R. Nygaard
Mycogen Corporation
5451 Oberlin Drive
San Diego, CA 92121

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS
OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <u>Escherichia coli</u> NM 522/pNYC 1642 NR626	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NRRL B-18961
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
<p>The microorganism identified under I above was accompanied by:</p> <p><input type="checkbox"/> a scientific description</p> <p><input checked="" type="checkbox"/> a proposed taxonomic designation</p> <p>(Mark with a cross where applicable)</p>	
III. RECEIPT AND ACCEPTANCE	
<p>This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 4-10-92 (date of the original deposit)¹</p>	
IV. RECEIPT OF REQUEST FOR CONVERSION	
<p>The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)</p>	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
<p>Name: Agricultural Research Culture Collection (NRRL) International Depositary Authority Address: 1815 N. University Street Peoria, Illinois 61604 U.S.A.</p>	<p>Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: <u>4-16-92</u></p>

Where Rule 6.4(d) applies, such date is the date on which the status of international depositary
authority was acquired.

Form EP/1 (sole page)

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Schnepf, Harry E.
Schwab, George E.
Payne, Jewel M.
Narva, Kenneth E.
Foncerrada, Luis
- (ii) TITLE OF INVENTION: Novel Nematode-Active Toxins and Genes
Which Code Therefor
- (iii) NUMBER OF SEQUENCES: 40
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: David R. Saliwanchik
(B) STREET: 2421 N.W. 41st Street, Suite A-1
(C) CITY: Gainesville
(D) STATE: FL
(E) COUNTRY: USA
(F) ZIP: 32606
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Saliwanchik, David R.
(B) REGISTRATION NUMBER: 31,794
(C) REFERENCE/DOCKET NUMBER: MA20C2C1C1
- (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 904-375-8100
(B) TELEFAX: 904-372-5800

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4155 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Bacillus thuringiensis
(B) STRAIN: PS17
(C) INDIVIDUAL ISOLATE: PS17a
- (vii) IMMEDIATE SOURCE:
(B) CLONE: E. coli NM522(pMYC 1627) NRRL B-18651

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGCAATTT TAAATGAATT ATATCCATCT GTACCTTATA ATGTATTGGC GTATACGCCA	60
CCCTCTTTTT TACCTGATGC GGGTACACAA GCTACACCTG CTGACTTAAC AGCTTATGAA	120
CAATTGTTGA AAAATTTAGA AAAAGGGATA AATGCTGGAA CTTATTTCGAA AGCAATAGCT	180
GATGTACTTA AAGGTATTTT TATAGATGAT ACAATAAATT ATCAAACATA TGTAATATT	240
GGTTTAAGTT TAATTACATT AGCTGTACCG GAAATTGGTA TTTTACACC TTTCATCGGT	300
TTGTTTTTTG CTGCATTGAA TAAACATGAT GCTCCACCTC CTCCTAATGC AAAAGATATA	360
TTTGAGGCTA TGAAACCAGC GATTCAAGAG ATGATTGATA GAACTTTAAC TCGGATGAG	420
CAAACATTTT TAAATGGGGA AATAAGTGGT TTACAAAATT TAGCAGCAAG ATACCACTCT	480
ACAATGGATG ATATTCAAAG CCATGGAGGA TTTAATAAGG TAGATTCTGG ATTAATTAA	540

AAGTTTACAG	ATGAGGTACT	ATCTTTAAAT	AGTTTTTATA	CAGATCGTTT	ACCTGTATTT	600
ATTACAGATA	ATACAGCGGA	TCGAACTTTG	TTAGGTCTTC	CTTATTATGC	TATACTTGCG	660
AGCATGCATC	TTATGTTATT	AAGAGATATC	ATTACTAAGG	GTCCGACATG	GGATTCTAAA	720
ATTAATTTCA	CACCAGATGC	AATTGATTCC	TTTAAAACCG	ATATTAAAAA	TAATATAAAG	780
CTTTACTCTA	AAACTATTTA	TGACGTATTT	CAGAAGGGAC	TTGCTTCATA	CGGAACGCCT	840
TCTGATTTAG	AGTCCTTTGC	AAAAAAACAA	AAATATATTG	AAATTATGAC	AACACATTGT	900
TTAGATTTTG	CAAGATTGTT	TCCTACTTTT	GATCCAGATC	TTTATCCAAC	AGGATCAGGT	960
GATATAAGTT	TACAAAAAAC	ACGTAGAATT	CTTCTCCTT	TTATCCCTAT	ACGTACTGCA	1020
GATGGGTAA	CATTAAATAA	TACTTCAATT	GATACTTCAA	ATTGGCCTAA	TTATGAAAAT	1080
GGGAATGGCG	CGTTTCCAAA	CCCAAAAGAA	AGAATATTAA	AACAATTCAA	ACTGTATCCT	1140
AGTTGGAGAG	CGGGACAGTA	CGGTGGGCTT	TTACAACCTT	ATTTATGGGC	AATAGAAGTC	1200
CAAGATTCTG	TAGAGACTCG	TTTGTATGGG	CAGCTTCCAG	CTGTAGATCC	ACAGGCAGGG	1260
CCTAATTATG	TTTCCATAGA	TTCTTCTAAT	CCAATCATAC	AAATAAATAT	GGATACTTGG	1320
AAAACACCAC	CACAAGGTGC	GAGTGGGTGG	AATACAAATT	TAATGAGAGG	AAGTGTAAGC	1380
GGGTAAAGTT	TTTTACAACG	AGATGGTACG	AGACTTAGTG	CTGGTATGGG	TGGTGGTTTT	1440
GCTGATACAA	TATATAGTCT	CCCTGCAACT	CATTATCTTT	CTTATCTCTA	TGGAACTCCT	1500
TATCAAACCT	CTGATAACTA	TTCTGGTCAC	GTTGGTGCAT	TGGTAGGTGT	GAGTACGCCT	1560
CAAGAGGCTA	CTCTTCCTAA	TATTATAGGT	CAACCAGATG	AACAGGGAAA	TGTATCTACA	1620
ATGGGATTTT	CGTTTGAAAA	AGCTTCTTAT	GGAGGTACAG	TTGTTAAAGA	ATGGTTAAAT	1680
GGTGCGAATG	CGATGAAGCT	TTCTCCTGGG	CAATCTATAG	GTATTCCTAT	TACAAATGTA	1740
ACAAGTGGAG	AATATCAAAT	TCGTTGTCGT	TATGCAAGTA	ATGATAATAC	TAACGTTTTT	1800
TTTAATGTAG	ATACTGGTGG	AGCAAATCCA	ATTTTCCAAC	AGATAAACTT	TGCATCTACT	1860
GTAGATAATA	ATACGGGAGT	ACAAGGAGCA	AATGGTGTCT	ATGTAGTCAA	ATCTATTGCT	1920
ACAACCTGATA	ATTCTTTTAC	AGAAATTCCT	GCGAAGACGA	TTAATGTTCA	TTTAACCAAC	1980
CAAGGTTCTT	CTGATGTCTT	TTTAGACCGT	ATTGAATTTA	TACCTTTTTT	TCTACCTCTT	2040
ATATATCATG	GAAGTTATAA	TACTTCATCA	GGTGCAGATG	ATGTTTTATG	GTCTTCTTCA	2100
AATATGAATT	ACTACGATAT	AATAGTAAAT	GGTCAGGCCA	ATAGTAGTAG	TATCGCTAGT	2160
TCTATGCATT	TGCTTAATAA	AGGAAAAGTG	ATAAAAACAA	TTGATATTCC	AGGGCATTCC	2220
GAAACCTTCT	TTGCTACGTT	CCCAGTTCCA	GAAGGATTTA	ATGAAGTTAG	AATTCTTGCT	2280
GGCCTTCCAG	AAGTTAGTGG	AAATATTACC	GTACAATCTA	ATAATCCGCC	TCAACCTAGT	2340
AATAATGGTG	GTGGTGATGG	TGGTGGTAAT	GGTGGTGGTG	ATGGTGGTCA	ATACAATTTT	2400
TCTTTAAGCG	GATCTGATCA	TACGACTATT	TATCATGGAA	AACTTGAAAC	TGGGATTTCAT	2460
GTACAAGGTA	ATTATACCTA	TACAGGTACT	CCCGTATTAA	TACTGAATGC	TTACAGAAAT	2520
AATACTGTAG	TATCAAGCAT	TCCAGTATAT	TCTCCTTTTG	ATATAACTAT	ACAGACAGAA	2580
GCTGATAGCC	TTGAGCTTGA	ACTACAACCT	AGATATGGTT	TTGCCACAGT	GAATGGTACT	2640
GCAACAGTAA	AAAGTCCTAA	TGTAAATTAC	GATAGATCAT	TTAAACTCCC	AATAGACTTA	2700
CAAAATATCA	CAACACAAGT	AAATGCATTA	TTCGCATCTG	GAACACAAAA	TATGCTTGCT	2760
CATAATGTAA	GTGATCATGA	TATTGAAGAA	GTTGTATTAA	AAGTGGATGC	CTTATCAGAT	2820
GAAGTATTTG	GAGATGAGAA	GAAGGCTTTA	CGTAAATTGG	TGAATCAAGC	AAAACGTTTG	2880
AGTAGAGCAA	GAAATCTTCT	GATAGGTGGG	AGTTTTGAAA	ATTGGGATGC	ATGGTATAAA	2940
GGAAGAAATG	TAGTAACTGT	ATCTGATCAT	GAACATTTTA	AGAGTGATCA	TGTATTATTA	3000
CCACCACCAG	GATTGTCTCC	ATCTTATATT	TTCCAAAAG	TGGAGGAATC	TAAATTAAAA	3060
CCAAATACAC	GTTATATTGT	TTCTGGATTC	ATCGCACATG	GAAAAGACCT	AGAAATTGTT	3120
GTTTCACGTT	ATGGGCAAGA	AGTGCAAAAG	GTCGTGCAAG	TTCCTTATGG	AGAAGCATTC	3180

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CCGTTAACAT CAAATGGACC AGTTTGTGTG CCCCCACGTT CTACAAGTAA TGGAACCTTA 3240
 GGAGATCCAC ATTTCTTTAG TTACAGTATC GATGTAGGTG CACTAGATTT ACAAGCAAAC 3300
 CCTGGTATTG AATTGGTCT TCGTATTGTA AATCCAAC TGATGGGCACG CGTAAGCAAT 3360
 TTGGAAATTC GTGAAGATCG TCCATTAGCA GCAAATGAAA TACGACAAGT ACAACGTGTC 3420
 GCAAGAAATT GGAGAACCGA GTATGAGAAA GAACGTGCGG AAGTAACAAG TTTAATTCAA 3480
 CCTGTTATCA ATCGAATCAA CGGATTGTAT GAAAATGGAA ATTGGAACGG TTCTATTTCGT 3540
 TCAGATATTT CGTATCAGAA TATAGACGCG ATTGTATTAC CAACGTTACC AAAGTTACGC 3600
 CATTGGTTTA TGTCAGATAG ATTCAGTGAA CAAGGAGATA TAATGGCTAA ATTCCAAGGT 3660
 GCATTAAATC GTGCGTATGC ACAACTGGAA CAAAGTACGC TTCTGCATAA TGGTCATTTT 3720
 ACAAAGATG CAGCTAATTG GACAATAGAA GGCGATGCAC ATCAGATAAC ACTAGAAGAT 3780
 GGTAGACGTG TATTGCGACT TCCAGATTGG TCTTCGAGTG TATCTCAAAT GATTGAAATC 3840
 GAGAATTTTA ATCCAGATAA AGAATACAAC TTAGTATTCC ATGGGCAAGG AGAAGGAACG 3900
 GTTACGTTGG AGCATGGAGA AGAAACAAAA TATATAGAAA CGCATACACA TCATTTTTCG 3960
 AATTTTACAA CTTCTCAACG TCAAGGACTC ACGTTTGAAT CAAATAAAGT GACAGTGACC 4020
 ATTTCTTCAG AAGATGGAGA ATTCTTAGTG GATAATATTG CGCTTGTTGA AGCTCCTCTT 4080
 CCTACAGATG ACCAAAATTC TGAGGGAAAT ACGGCTTCCA GTACGAATAG CGATACAAGT 4140
 ATGAACAACA ATCAA 4155

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1385 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: BACILLUS THURINGIENSIS
- (C) INDIVIDUAL ISOLATE: PS17

(vii) IMMEDIATE SOURCE:

- (B) CLONE: E. coli NM522(pMYC 1627) NRRL B-18651

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Ile Leu Asn Glu Leu Tyr Pro Ser Val Pro Tyr Asn Val Leu
 1 5 10
 Ala Tyr Thr Pro Pro Ser Phe Leu Pro Asp Ala Gly Thr Gln Ala Thr
 20 25 30
 Pro Ala Asp Leu Thr Ala Tyr Glu Gln Leu Leu Lys Asn Leu Glu Lys
 35 40 45
 Gly Ile Asn Ala Gly Thr Tyr Ser Lys Ala Ile Ala Asp Val Leu Lys
 50 55 60
 Gly Ile Phe Ile Asp Asp Thr Ile Asn Tyr Gln Thr Tyr Val Asn Ile
 65 70 75 80
 Gly Leu Ser Leu Ile Thr Leu Ala Val Pro Glu Ile Gly Ile Phe Thr
 85 90 95
 Pro Phe Ile Gly Leu Phe Phe Ala Ala Leu Asn Lys His Asp Ala Pro
 100 105 110
 Pro Pro Pro Asn Ala Lys Asp Ile Phe Glu Ala Met Lys Pro Ala Ile
 115 120 125
 Gln Glu Met Ile Asp Arg Thr Leu Thr Ala Asp Glu Gln Thr Phe Leu
 130 135 140

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Asn Gly Glu Ile Ser Gly Leu Gln Asn Leu Ala Ala Arg Tyr Gln Ser
 145 150 155 160
 Thr Met Asp Asp Ile Gln Ser His Gly Gly Phe Asn Lys Val Asp Ser
 165 170 175
 Gly Leu Ile Lys Lys Phe Thr Asp Glu Val Leu Ser Leu Asn Ser Phe
 180 185 190
 Tyr Thr Asp Arg Leu Pro Val Phe Ile Thr Asp Asn Thr Ala Asp Arg
 195 200 205
 Thr Leu Leu Gly Leu Pro Tyr Tyr Ala Ile Leu Ala Ser Met His Leu
 210 215 220
 Met Leu Leu Arg Asp Ile Ile Thr Lys Gly Pro Thr Trp Asp Ser Lys
 225 230 235 240
 Ile Asn Phe Thr Pro Asp Ala Ile Asp Ser Phe Lys Thr Asp Ile Lys
 245 250 255
 Asn Asn Ile Lys Leu Tyr Ser Lys Thr Ile Tyr Asp Val Phe Gln Lys
 260 265 270
 Gly Leu Ala Ser Tyr Gly Thr Pro Ser Asp Leu Glu Ser Phe Ala Lys
 275 280 285
 Lys Gln Lys Tyr Ile Glu Ile Met Thr Thr His Cys Leu Asp Phe Ala
 290 295 300
 Arg Leu Phe Pro Thr Phe Asp Pro Asp Leu Tyr Pro Thr Gly Ser Gly
 305 310 315 320
 Asp Ile Ser Leu Gln Lys Thr Arg Arg Ile Leu Ser Pro Phe Ile Pro
 325 330 335
 Ile Arg Thr Ala Asp Gly Leu Thr Leu Asn Asn Thr Ser Ile Asp Thr
 340 345 350
 Ser Asn Trp Pro Asn Tyr Glu Asn Gly Asn Gly Ala Phe Pro Asn Pro
 355 360 365
 Lys Glu Arg Ile Leu Lys Gln Phe Lys Leu Tyr Pro Ser Trp Arg Ala
 370 375 380
 Gly Gln Tyr Gly Gly Leu Leu Gln Pro Tyr Leu Trp Ala Ile Glu Val
 385 390 395 400
 Gln Asp Ser Val Glu Thr Arg Leu Tyr Gly Gln Leu Pro Ala Val Asp
 405 410 415
 Pro Gln Ala Gly Pro Asn Tyr Val Ser Ile Asp Ser Ser Asn Pro Ile
 420 425 430
 Ile Gln Ile Asn Met Asp Thr Trp Lys Thr Pro Pro Gln Gly Ala Ser
 435 440 445
 Gly Trp Asn Thr Asn Leu Met Arg Gly Ser Val Ser Gly Leu Ser Phe
 450 455 460
 Leu Gln Arg Asp Gly Thr Arg Leu Ser Ala Gly Met Gly Gly Gly Phe
 465 470 475 480
 Ala Asp Thr Ile Tyr Ser Leu Pro Ala Thr His Tyr Leu Ser Tyr Leu
 485 490 495
 Tyr Gly Thr Pro Tyr Gln Thr Ser Asp Asn Tyr Ser Gly His Val Gly
 500 505 510
 Ala Leu Val Gly Val Ser Thr Pro Gln Glu Ala Thr Leu Pro Asn Ile
 515 520 525
 Ile Gly Gln Pro Asp Glu Gln Gly Asn Val Ser Thr Met Gly Phe Pro
 530 535 540
 Phe Glu Lys Ala Ser Tyr Gly Gly Thr Val Val Lys Glu Trp Leu Asn
 545 550 555 560
 Gly Ala Asn Ala Met Lys Leu Ser Pro Gly Gln Ser Ile Gly Ile Pro
 565 570 575
 Ile Thr Asn Val Thr Ser Gly Glu Tyr Gln Ile Arg Cys Arg Tyr Ala
 580 585 590
 Ser Asn Asp Asn Thr Asn Val Phe Phe Asn Val Asp Thr Gly Gly Ala
 595 600 605

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Asn Pro Ile Phe Gln Gln Ile Asn Phe Ala Ser Thr Val Asp Asn Asn
 610 615 620
 Thr Gly Val Gln Gly Ala Asn Gly Val Tyr Val Val Lys Ser Ile Ala
 625 630 635 640
 Thr Thr Asp Asn Ser Phe Thr Glu Ile Pro Ala Lys Thr Ile Asn Val
 645 650 655
 His Leu Thr Asn Gln Gly Ser Ser Asp Val Phe Leu Asp Arg Ile Glu
 660 665 670
 Phe Ile Pro Phe Ser Leu Pro Leu Ile Tyr His Gly Ser Tyr Asn Thr
 675 680 685
 Ser Ser Gly Ala Asp Asp Val Leu Trp Ser Ser Ser Asn Met Asn Tyr
 690 695 700
 Tyr Asp Ile Ile Val Asn Gly Gln Ala Asn Ser Ser Ser Ile Ala Ser
 705 710 715 720
 Ser Met His Leu Leu Asn Lys Gly Lys Val Ile Lys Thr Ile Asp Ile
 725 730 735
 Pro Gly His Ser Glu Thr Phe Phe Ala Thr Phe Pro Val Pro Glu Gly
 740 745 750
 Phe Asn Glu Val Arg Ile Leu Ala Gly Leu Pro Glu Val Ser Gly Asn
 755 760 765
 Ile Thr Val Gln Ser Asn Asn Pro Pro Gln Pro Ser Asn Asn Gly Gly
 770 775 780
 Gly Asp Gly Gly Gly Asn Gly Gly Gly Asp Gly Gly Gln Tyr Asn Phe
 785 790 795 800
 Ser Leu Ser Gly Ser Asp His Thr Thr Ile Tyr His Gly Lys Leu Glu
 805 810 815
 Thr Gly Ile His Val Gln Gly Asn Tyr Thr Tyr Thr Gly Thr Pro Val
 820 825 830
 Leu Ile Leu Asn Ala Tyr Arg Asn Asn Thr Val Val Ser Ser Ile Pro
 835 840 845
 Val Tyr Ser Pro Phe Asp Ile Thr Ile Gln Thr Glu Ala Asp Ser Leu
 850 855 860
 Glu Leu Glu Leu Gln Pro Arg Tyr Gly Phe Ala Thr Val Asn Gly Thr
 865 870 875 880
 Ala Thr Val Lys Ser Pro Asn Val Asn Tyr Asp Arg Ser Phe Lys Leu
 885 890 895
 Pro Ile Asp Leu Gln Asn Ile Thr Thr Gln Val Asn Ala Leu Phe Ala
 900 905 910
 Ser Gly Thr Gln Asn Met Leu Ala His Asn Val Ser Asp His Asp Ile
 915 920 925
 Glu Glu Val Val Leu Lys Val Asp Ala Leu Ser Asp Glu Val Phe Gly
 930 935 940
 Asp Glu Lys Lys Ala Leu Arg Lys Leu Val Asn Gln Ala Lys Arg Leu
 945 950 955 960
 Ser Arg Ala Arg Asn Leu Leu Ile Gly Gly Ser Phe Glu Asn Trp Asp
 965 970 975
 Ala Trp Tyr Lys Gly Arg Asn Val Val Thr Val Ser Asp His Glu Leu
 980 985 990
 Phe Lys Ser Asp His Val Leu Leu Pro Pro Pro Gly Leu Ser Pro Ser
 995 1000 1005
 Tyr Ile Phe Gln Lys Val Glu Glu Ser Lys Leu Lys Pro Asn Thr Arg
 1010 1015 1020
 Tyr Ile Val Ser Gly Phe Ile Ala His Gly Lys Asp Leu Glu Ile Val
 1025 1030 1035 1040
 Val Ser Arg Tyr Gly Gln Glu Val Gln Lys Val Val Gln Val Pro Tyr
 1045 1050 1055
 Gly Glu Ala Phe Pro Leu Thr Ser Asn Gly Pro Val Cys Cys Pro Pro
 1060 1065 1070

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Arg Ser Thr Ser Asn Gly Thr Leu Gly Asp Pro His Phe Phe Ser Tyr
 1075 1080 1085
 Ser Ile Asp Val Gly Ala Leu Asp Leu Gln Ala Asn Pro Gly Ile Glu
 1090 1095 1100
 Phe Gly Leu Arg Ile Val Asn Pro Thr Gly Met Ala Arg Val Ser Asn
 1105 1110 1115 1120
 Leu Glu Ile Arg Glu Asp Arg Pro Leu Ala Ala Asn Glu Ile Arg Gln
 1125 1130 1135
 Val Gln Arg Val Ala Arg Asn Trp Arg Thr Glu Tyr Glu Lys Glu Arg
 1140 1145 1150
 Ala Glu Val Thr Ser Leu Ile Gln Pro Val Ile Asn Arg Ile Asn Gly
 1155 1160 1165
 Leu Tyr Glu Asn Gly Asn Trp Asn Gly Ser Ile Arg Ser Asp Ile Ser
 1170 1175 1180
 Tyr Gln Asn Ile Asp Ala Ile Val Leu Pro Thr Leu Pro Lys Leu Arg
 1185 1190 1195 1200
 His Trp Phe Met Ser Asp Arg Phe Ser Glu Gln Gly Asp Ile Met Ala
 1205 1210 1215
 Lys Phe Gln Gly Ala Leu Asn Arg Ala Tyr Ala Gln Leu Glu Gln Ser
 1220 1225 1230
 Thr Leu Leu His Asn Gly His Phe Thr Lys Asp Ala Ala Asn Trp Thr
 1235 1240 1245
 Ile Glu Gly Asp Ala His Gln Ile Thr Leu Glu Asp Gly Arg Arg Val
 1250 1255 1260
 Leu Arg Leu Pro Asp Trp Ser Ser Ser Val Ser Gln Met Ile Glu Ile
 1265 1270 1275 1280
 Glu Asn Phe Asn Pro Asp Lys Glu Tyr Asn Leu Val Phe His Gly Gln
 1285 1290 1295
 Gly Glu Gly Thr Val Thr Leu Glu His Gly Glu Glu Thr Lys Tyr Ile
 1300 1305 1310
 Glu Thr His Thr His His Phe Ala Asn Phe Thr Thr Ser Gln Arg Gln
 1315 1320 1325
 Gly Leu Thr Phe Glu Ser Asn Lys Val Thr Val Thr Ile Ser Ser Glu
 1330 1335 1340
 Asp Gly Glu Phe Leu Val Asp Asn Ile Ala Leu Val Glu Ala Pro Leu
 1345 1350 1355 1360
 Pro Thr Asp Asp Gln Asn Ser Glu Gly Asn Thr Ala Ser Ser Thr Asn
 1365 1370 1375
 Ser Asp Thr Ser Met Asn Asn Asn Gln
 1380 1385

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3867 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Bacillus thuringiensis*
 - (B) STRAIN: PS17
 - (C) INDIVIDUAL ISOLATE: PS17b
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: E. coli NM522(pMYC 1628) NRRL B-18652

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGCAATTT TAAATGAATT ATATCCATCT GTACCTTATA ATGTATTGGC GTATACGCCA

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CCCTCTTTTT TACCTGATGC GGGTACACAA GCTACACCTG CTGACTTAAC AGCTTATGAA	120
CAATTGTTGA AAAATTTAGA AAAAGGGATA AATGCTGGAA CTTATTCGAA AGCAATAGCT	180
GATGTACTTA AAGGTATTTT TATAGATGAT ACAATAAATT ATCAAACATA TGTAATATT	240
GGTTTAAGTT TAATTACATT AGCTGTACCG GAAATTGGTA TTTTACACC TTTCATCGGT	300
TTGTTTTTGT CTGCATTGAA TAAACATGAT GCTCCACCTC CTCCTAATGC AAAAGATATA	360
TTTGAGGCTA TGAAACCAGC GATTCAAGAG ATGATTGATA GAACTTTAAC TGC GGATGAG	420
CAAACATTTT TAAATGGGGA AATAAGTGGT TTACAAAATT TAGCAGCAAG ATACCAGTCT	480
ACAATGGATG ATATTCAAAG CCATGGAGGA TTTAATAAGG TAGATTCTGG ATTAATTAAA	540
AAGTTTACAG ATGAGGTACT ATCTTTAAAT AGTTTTTATA CAGATCGTTT ACCTGTATTT	600
ATTACAGATA ATACAGCGGA TCGAACTTTG TTAGGTCTTC CTTATTATGC TATACTTGCG	660
AGCATGCATC TTATGTTATT AAGAGATATC ATTACTAAGG GTCCGACATG GGATTCTAAA	720
ATTAATTCA CACCAGATGC AATTGATTCC TTTAAAACCG ATATTAAAAA TAATATAAAG	780
CTTACTCTA AAACATTTTA TGACGTATTT CAGAAGGGAC TTGCTTCATA CGGAACGCCCT	840
TCTGATTAG AGTCCTTTGC AAAAAACAA AAATATATTG AAATTATGAC AACACATTGT	900
TTAGATTTTG CAAGATTGTT TCCTACTTTT GATCCAGATC TTTATCCAAC AGGATCAGGT	960
GATATAAGTT TACAAAAAAC ACGTAGAATT CTTTCTCCTT TTATCCCTAT ACGTACTGCA	1020
GATGGGTTAA CATTAAATAA TACTTCAATT GATACTTCAA ATTGGCCTAA TTATGAAAAT	1080
GGGAATGGCG CGTTTCCAAA CCCAAAAGAA AGAATATTAA AACAAATCAA ACTGTATCCT	1140
AGTTGGAGAG CGGCACAGTA CGGTGGGCTT TTACAACCTT ATTTATGGGC AATAGAAGTC	1200
CAAGATTCTG TAGAGACTCG TTTGTATGGG CAGCTTCCAG CTGTAGATCC ACAGGCAGGG	1260
CCTAATTATG TTTCCATAGA TTCTTCTAAT CCAATCATAC AAATAAATAT GGATACTTGG	1320
AAAACACCAC CACAAGGTGC GAGTGGGTGG AATACAAATT TAATGAGAGG AAGTGTAAGC	1380
GGGTTAAGTT TTTTACAACG AGATGGTACG AGACTTAGTG CTGGTATGGG TGGTGGTTTT	1440
GCTGATACAA TATATAGTCT CCCTGCAACT CATTATCTTT CTTATCTCTA TGGAACTCCT	1500
TATCAAACCT CTGATAACTA TTCTGGTCAC GTTGGTGCAT TGGTAGGTGT GAGTACGCCT	1560
CAAGAGGCTA CTCTTCCTAA TATTATAGGT CAACCAGATG AACAGGGAAA TGTATCTACA	1620
ATGGGATTTT CGTTTGAAAA AGCTTCTTAT GGAGGTACAG TTGTTAAAGA ATGGTTAAAT	1680
GGTGC GAATG CGATGAAGCT TTCTCCTGGG CAATCTATAG GTATTCTCTAT TACAAATGTA	1740
ACAAGTGGAG AATATCAAAT TCGTTGTCGT TATGCAAGTA ATGATAATAC TAACGTTTTT	1800
TTTAATGTAG ATACTGGTGG AGCAAATCCA ATTTTCCAAC AGATAAACTT TGCATCTACT	1860
GTAGATAATA ATACGGGAGT ACAAGGAGCA AATGGTGTCT ATGTAGTCAA ATCTATTGCT	1920
ACAACCTGATA ATCTTTTAC AGTAAAAATT CCTGCGAAGA CGATTAATGT TCATTTAACC	1980
AACCAAGGTT CTTCTGATGT CTTTTTAGAT CGTATTGAGT TTGTTCCAAT TCTAGAATCA	2040
AATACTGTAA CTATATTCAA CAATTCATAT ACTACAGGTT CAGCAAATCT TATACCAGCA	2100
ATAGCTCCTC TTTGGAGTAC TAGTTCAGAT AAAGCCCTTA CAGGTTCTAT GTCAATAACA	2160
GGTCGAACCTA CCCCTAACAG TGATGATGCT TTGCTTCGAT TTTTAAAC TAATTATGAT	2220
ACACAAACCA TTCCTATTCC GGGTTCGGA AAAGATTTTA CAAATACTCT AGAAATACAA	2280
GACATAGTTT CTATTGATAT TTTTGTGCGA TCTGGTCTAC ATGGATCCGA TGGATCTATA	2340
AAATTAGATT TTACCAATAA TAATAGTGGT AGTGGTGGCT CTCCAAAGAG TTTCACCGAG	2400
CAAAATGATT TAGAGAATAT CACAACACAA GTGAATGCTC TATTCACATC TAATACACAA	2460
GATGCACCTG CAACAGATGT GAGTGATCAT GATATTGAAG AAGTGGTTCT AAAAGTAGAT	2520
GCATTATCTG ATGAAGTGT TGGAAAAGAG AAAAAACAT TGCGTAAATT TGTAAATCAA	2580
GCGAAGCGCT TAAGCAAGGC GCGTAATCTC CTGGTAGGAG GCAATTTTGA TAACTTGGAT	2640
GCTTGGTATA GAGGAAGAAA TGTAGTAAAC GTATCTAATC ACGAACTGTT GAAGAGTGAT	2700

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CATGTATTAT TACCACCACC AGGATTGTCT CCATCTTATA TTTTCCAAAA AGTGGAGGAA 2760
 TCTAAATTAA AACGAAATAC ACGTTATACG GTTTCTGGAT TTATTGCGCA TGCAACAGAT 2820
 TTAGAAATTG TGGTTTCTCG TTATGGGCAA GAAATAAAGA AAGTGGTGCA AGTTCCTTAT 2880
 GGAGAAGCAT TCCCATTAAAC ATCAAGTGGA CCAGTTTGTT GTATCCCACA TTCTACAAGT 2940
 AATGGAACCT TAGGCAATCC ACATTTCTTT AGTTACAGTA TTGATGTAGG TGCATTAGAT 3000
 GTAGACACAA ACCCTGGTAT TGAATTCGGT CTTCTGATTG TAAATCCAAC TGGAATGGCA 3060
 CGCGTAAGCA ATTTGGAAAT TCGTGAAGAT CGTCCATTAG CAGCAAATGA AATACGACAA 3120
 GTACAACGTG TCGCAAGAAA TTGGAGAACC GAGTATGAGA AAGAACGTGC GGAAGTAACA 3180
 AGTTTAATTC AACCTGTTAT CAATCGAATC AATGGATTGT ATGACAATGG AAATTGGAAC 3240
 GGTTCATTTC GTTCAGATAT TTCGTATCAG AATATAGACG CGATTGTATT ACCAACGTTA 3300
 CCAAAGTTAC GCCATTGGTT TATGTCAGAT AGATTTAGTG AACAAAGGAGA TATCATGGCT 3360
 AAATTCCAAG GTGCATTAAA TCGTGCCTAT GCACAACCTGG AACAAAATAC GCTTCTGCAT 3420
 AATGGTCATT TTACAAAAGA TGCAGCCAAT TGGACGGTAG AAGGCGATGC ACATCAGGTA 3480
 GTATTAGAAG ATGGTAAACG TGTATTACGA TTGCCAGATT GGTCTTCGAG TGTGTCTCAA 3540
 ACGATTGAAA TCGAGAATTT TGATCCAGAT AAAGAATATC AATTAGTATT TCATGGGCAA 3600
 GGAGAAGGAA CGGTTACGTT GGAGCATGGA GAAGAAACAA AATATATAGA AACGCATACA 3660
 CATCATTTTG CGAATTTTAC AACTTCTCAA CGTCAAGGAC TCACGTTTGA ATCAAATAAA 3720
 GTGACAGTGA CCATTTCTTC AGAAGATGGA GAATTCTTAG TGGATAATAT TCGCCTTG TG 3780
 GAAGCTCCTC TTCCTACAGA TGACCAAAAT TCTGAGGGAA ATACGGCTTC CAGTACGAAT 3840
 AGCGATACAA GTATGAACAA CAATCAA 3867

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1289 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: BACILLUS THURINGIENSIS
 - (C) INDIVIDUAL ISOLATE: PS17
- (vii) IMMEDIATE SOURCE:
- (B) CLONE: E. coli NM522(pMYC 1628) NRRL B-18652
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
- | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ala | Ile | Leu | Asn | Glu | Leu | Tyr | Pro | Ser | Val | Pro | Tyr | Asn | Val | Leu |
| 1 | | | | 5 | | | | | 10 | | | | 15 | | |
| Ala | Tyr | Thr | Pro | Pro | Ser | Phe | Leu | Pro | Asp | Ala | Gly | Thr | Gln | Ala | Thr |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Pro | Ala | Asp | Leu | Thr | Ala | Tyr | Glu | Gln | Leu | Leu | Lys | Asn | Leu | Glu | Lys |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Gly | Ile | Asn | Ala | Gly | Thr | Tyr | Ser | Lys | Ala | Ile | Ala | Asp | Val | Leu | Lys |
| | 50 | | | | | 55 | | | | 60 | | | | | |
| Gly | Ile | Phe | Ile | Asp | Asp | Thr | Ile | Asn | Tyr | Gln | Thr | Tyr | Val | Asn | Ile |
| | 65 | | | | 70 | | | | | 75 | | | | 80 | |
| Gly | Leu | Ser | Leu | Ile | Thr | Leu | Ala | Val | Pro | Glu | Ile | Gly | Ile | Phe | Thr |
| | | | 85 | | | | | 90 | | | | | | 95 | |
| Pro | Phe | Ile | Gly | Leu | Phe | Phe | Ala | Ala | Leu | Asn | Lys | His | Asp | Ala | Pro |
| | | | 100 | | | | 105 | | | | | | 110 | | |

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Pro Pro Pro₁₁₅ Asn Ala Lys Asp Ile₁₂₀ Phe Glu Ala Met Lys₁₂₅ Pro Ala Ile
 Gln Glu₁₃₀ Met Ile Asp Arg Thr₁₃₅ Leu Thr Ala Asp Glu₁₄₀ Gln Thr Phe Leu
 Asn Gly Glu Ile Ser₁₅₀ Gly Leu Gln Asn Leu Ala₁₅₅ Ala Arg Tyr Gln Ser₁₆₀
 Thr Met Asp Asp Ile₁₆₅ Gln Ser His Gly₁₇₀ Gly Phe Asn Lys Val Asp Ser₁₇₅
 Gly Leu Ile Lys₁₈₀ Lys Phe Thr Asp Glu₁₈₅ Val Leu Ser Leu Asn Ser Phe₁₉₀
 Tyr Thr Asp₁₉₅ Arg Leu Pro Val Phe₂₀₀ Ile Thr Asp Asn Thr₂₀₅ Ala Asp Arg
 Thr Leu Leu Gly Leu Pro Tyr₂₁₅ Tyr Ala Ile Leu Ala₂₂₀ Ser Met His Leu
 Met Leu Leu Arg Asp Ile₂₃₀ Ile Thr Lys Gly Pro₂₃₅ Thr Trp Asp Ser Lys₂₄₀
 Ile Asn Phe Thr Pro₂₄₅ Asp Ala Ile Asp Ser₂₅₀ Phe Lys Thr Asp Ile Lys₂₅₅
 Asn Asn Ile Lys₂₆₀ Leu Tyr Ser Lys Thr₂₆₅ Ile Tyr Asp Val Phe Gln Lys₂₇₀
 Gly Leu Ala Ser Tyr Gly Thr Pro₂₈₀ Ser Asp Leu Glu Ser₂₈₅ Phe Ala Lys
 Lys Gln Lys Tyr Ile Glu Ile₂₉₅ Met Thr Thr His Cys₃₀₀ Leu Asp Phe Ala
 Arg Leu Phe Pro Thr Phe₃₁₀ Asp Pro Asp Leu Tyr₃₁₅ Pro Thr Gly Ser Gly₃₂₀
 Asp Ile Ser Leu Gln Lys Thr Arg Arg Ile₃₃₀ Leu Ser Pro Phe Ile Pro₃₃₅
 Ile Arg Thr Ala Asp Gly Leu Thr Leu₃₄₅ Asn Asn Thr Ser Ile Asp Thr₃₅₀
 Ser Asn Trp₃₅₅ Pro Asn Tyr Glu Asn₃₆₀ Gly Asn Gly Ala Phe Pro Asn Pro₃₆₅
 Lys Glu Arg Ile Leu Lys Gln₃₇₅ Phe Lys Leu Tyr Pro₃₈₀ Ser Trp Arg Ala
 Ala Gln Tyr Gly Gly Leu Leu Gln Pro Tyr Leu₃₉₅ Trp Ala Ile Glu Val₄₀₀
 Gln Asp Ser Val Glu Thr Arg Leu Tyr Gly₄₁₀ Gln Leu Pro Ala Val Asp₄₁₅
 Pro Gln Ala Gly₄₂₀ Pro Asn Tyr Val Ser₄₂₅ Ile Asp Ser Ser Asn Pro Ile₄₃₀
 Ile Gln Ile Asn Met Asp Thr Trp₄₄₀ Lys Thr Pro Pro Gln Gly Ala Ser₄₄₅
 Gly Trp Asn Thr Asn Leu Met₄₅₅ Arg Gly Ser Val Ser₄₆₀ Gly Leu Ser Phe
 Leu Gln Arg Asp Gly Thr₄₇₀ Arg Leu Ser Ala Gly₄₇₅ Met Gly Gly Gly Phe₄₈₀
 Ala Asp Thr Ile Tyr₄₈₅ Ser Leu Pro Ala Thr₄₉₀ His Tyr Leu Ser Tyr₄₉₅ Leu
 Tyr Gly Thr Pro₅₀₀ Tyr Gln Thr Ser Asp₅₀₅ Asn Tyr Ser Gly His Val Gly₅₁₀
 Ala Leu Val Gly Val Ser Thr Pro₅₂₀ Gln Glu Ala Thr Leu₅₂₅ Pro Asn Ile
 Ile Gly Gln Pro Asp Glu Gln₅₃₅ Gly Asn Val Ser Thr₅₄₀ Met Gly Phe Pro
 Phe Glu Lys Ala Ser Tyr₅₅₀ Gly Gly Thr Val Val₅₅₅ Lys Glu Trp Leu Asn₅₆₀
 Gly Ala Asn Ala Met₅₆₅ Lys Leu Ser Pro Gly₅₇₀ Gln Ser Ile Gly Ile Pro₅₇₅

51

Ile Thr Asn Val Thr Ser Gly Glu Tyr Gln Ile Arg Cys Arg Tyr Ala
 580 585 590
 Ser Asn Asp Asn Thr Asn Val Phe Phe Asn Val Asp Thr Gly Gly Ala
 595 600 605
 Asn Pro Ile Phe Gln Gln Ile Asn Phe Ala Ser Thr Val Asp Asn Asn
 610 615 620
 Thr Gly Val Gln Gly Ala Asn Gly Val Tyr Val Lys Ser Ile Ala
 625 630 635 640
 Thr Thr Asp Asn Ser Phe Thr Val Lys Ile Pro Ala Lys Thr Ile Asn
 645 650 655
 Val His Leu Thr Asn Gln Gly Ser Ser Asp Val Phe Leu Asp Arg Ile
 660 665 670
 Glu Phe Val Pro Ile Leu Glu Ser Asn Thr Val Thr Ile Phe Asn Asn
 675 680 685
 Ser Tyr Thr Thr Gly Ser Ala Asn Leu Ile Pro Ala Ile Ala Pro Leu
 690 695 700
 Trp Ser Thr Ser Ser Asp Lys Ala Leu Thr Gly Ser Met Ser Ile Thr
 705 710 715 720
 Gly Arg Thr Thr Pro Asn Ser Asp Asp Ala Leu Leu Arg Phe Phe Lys
 725 730 735
 Thr Asn Tyr Asp Thr Gln Thr Ile Pro Ile Pro Gly Ser Gly Lys Asp
 740 745 750
 Phe Thr Asn Thr Leu Glu Ile Gln Asp Ile Val Ser Ile Asp Ile Phe
 755 760 765
 Val Gly Ser Gly Leu His Gly Ser Asp Gly Ser Ile Lys Leu Asp Phe
 770 775 780
 Thr Asn Asn Asn Ser Gly Ser Gly Gly Ser Pro Lys Ser Phe Thr Glu
 785 790 795 800
 Gln Asn Asp Leu Glu Asn Ile Thr Thr Gln Val Asn Ala Leu Phe Thr
 805 810 815
 Ser Asn Thr Gln Asp Ala Leu Ala Thr Asp Val Ser Asp His Asp Ile
 820 825 830
 Glu Glu Val Val Leu Lys Val Asp Ala Leu Ser Asp Glu Val Phe Gly
 835 840 845
 Lys Glu Lys Lys Thr Leu Arg Lys Phe Val Asn Gln Ala Lys Arg Leu
 850 855 860
 Ser Lys Ala Arg Asn Leu Leu Val Gly Gly Asn Phe Asp Asn Leu Asp
 865 870 875 880
 Ala Trp Tyr Arg Gly Arg Asn Val Val Asn Val Ser Asn His Glu Leu
 885 890 895
 Leu Lys Ser Asp His Val Leu Leu Pro Pro Pro Gly Leu Ser Pro Ser
 900 905 910
 Tyr Ile Phe Gln Lys Val Glu Glu Ser Lys Leu Lys Arg Asn Thr Arg
 915 920 925
 Tyr Thr Val Ser Gly Phe Ile Ala His Ala Thr Asp Leu Glu Ile Val
 930 935 940
 Val Ser Arg Tyr Gly Gln Glu Ile Lys Lys Val Val Gln Val Pro Tyr
 945 950 955 960
 Gly Glu Ala Phe Pro Leu Thr Ser Ser Gly Pro Val Cys Cys Ile Pro
 965 970 975
 His Ser Thr Ser Asn Gly Thr Leu Gly Asn Pro His Phe Phe Ser Tyr
 980 985 990
 Ser Ile Asp Val Gly Ala Leu Asp Val Asp Thr Asn Pro Gly Ile Glu
 995 1000 1005
 Phe Gly Leu Arg Ile Val Asn Pro Thr Gly Met Ala Arg Val Ser Asn
 1010 1015 1020
 Leu Glu Ile Arg Glu Asp Arg Pro Leu Ala Ala Asn Glu Ile Arg Gln
 1025 1030 1035 1040

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Val Gln Arg Val Ala Arg Asn Trp Arg Thr Glu Tyr Glu Lys Glu Arg
 1045 1050 1055
 Ala Glu Val Thr Ser Leu Ile Gln Pro Val Ile Asn Arg Ile Asn Gly
 1060 1065 1070
 Leu Tyr Asp Asn Gly Asn Trp Asn Gly Ser Ile Arg Ser Asp Ile Ser
 1075 1080 1085
 Tyr Gln Asn Ile Asp Ala Ile Val Leu Pro Thr Leu Pro Lys Leu Arg
 1090 1095 1100
 His Trp Phe Met Ser Asp Arg Phe Ser Glu Gln Gly Asp Ile Met Ala
 1105 1110 1115 1120
 Lys Phe Gln Gly Ala Leu Asn Arg Ala Tyr Ala Gln Leu Glu Gln Asn
 1125 1130 1135
 Thr Leu Leu His Asn Gly His Phe Thr Lys Asp Ala Ala Asn Trp Thr
 1140 1145 1150
 Val Glu Gly Asp Ala His Gln Val Val Leu Glu Asp Gly Lys Arg Val
 1155 1160 1165
 Leu Arg Leu Pro Asp Trp Ser Ser Val Ser Gln Thr Ile Glu Ile
 1170 1175 1180
 Glu Asn Phe Asp Pro Asp Lys Glu Tyr Gln Leu Val Phe His Gly Gln
 1185 1190 1195 1200
 Gly Glu Gly Thr Val Thr Leu Glu His Gly Glu Glu Thr Lys Tyr Ile
 1205 1210 1215
 Glu Thr His Thr His His Phe Ala Asn Phe Thr Thr Ser Gln Arg Gln
 1220 1225 1230
 Gly Leu Thr Phe Glu Ser Asn Lys Val Thr Val Thr Ile Ser Ser Glu
 1235 1240 1245
 Asp Gly Glu Phe Leu Val Asp Asn Ile Ala Leu Val Glu Ala Pro Leu
 1250 1255 1260
 Pro Thr Asp Asp Gln Asn Ser Glu Gly Asn Thr Ala Ser Ser Thr Asn
 1265 1270 1275 1280
 Ser Asp Thr Ser Met Asn Asn Asn Gln
 1285

(2) INFORMATION FOR SEQ ID NO:5 (PS33F2):

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3771 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Bacillus thuringiensis
 - (C) INDIVIDUAL ISOLATE: 33f2
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: E. coli NM522(pMYC 2316) B-18785
- (ix) FEATURE:
 - (A) NAME/KEY: misc feature
 - (B) LOCATION: 4..24
 - (D) OTHER INFORMATION: /function= "oligonucleotide hybridization probe"
 /product= "GCA/T ACA/T TTA AAT GAA GTA/T TAT"
 /standard name= "probe a"
 /note= "PProbe A"
- (ix) FEATURE:
 - (A) NAME/KEY: misc feature
 - (B) LOCATION: 13..33
 - (D) OTHER INFORMATION: /function= "oligonucleotide hybridization probe"
 /product= "AAT GAA GTA/T TAT CCA/T GTA/T AAT"
 /standard name= "Probe B"
 /label= pProbe-b
 /note= "probe b"

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGGCTACAC	TTAATGAAGT	ATATCCTGTG	AATTATAATG	TATTATCTTC	TGATGCTTTT	60
CAACAATTAG	ATACAACAGG	TTTTAAAAGT	AAATATGATG	AAATGATAAA	AGCATTGCGA	120
AAAAAATGGA	AAAAAGGGGC	AAAAGGAAAA	GACCTTTTAG	ATGTTGCATG	GACTTATATA	180
ACTACAGGAG	AAATTGACCC	TTTAAATGTA	ATTAAAGGTG	TTTTATCTGT	TATTAACCTTA	240
ATTCCTGAAG	TTGGTACTGT	GGCCTCTGCA	GCAAGTACTA	TTGTAAGTTT	TATTTGGCCT	300
AAAATATTTG	GAGATAAACC	AAATGCAAAA	AATATATTTG	AAGAGCTCAA	GCCTCAAATT	360
GAAGCATTAA	TTCAACAAGA	TATAACAAAC	TATCAAGATG	CAATTAATCA	AAAAAAATTT	420
GACAGTCTTC	AGAAAACAAT	TAATCTATAT	ACAGTAGCTA	TAGATAACAA	TGATTACGTA	480
ACAGCAAAAA	CGCAACTCGA	AAATCTAAAT	TCTATACTTA	CCTCAGATAT	CTCCATATTT	540
ATTCAGAAAG	GATATGAAAC	TGGAGGTTTA	CCTTATTATG	CTATGGTTGC	TAATGCTCAT	600
ATATTATTGT	TAAGAGACGC	TATAGTTAAT	GCAGAGAAAT	TAGGCTTTAG	TGATAAAGAA	660
GTAGACACAC	ATAAAAAATA	TATCAAAATG	ACAATACACA	ATCATACTGA	AGCAGTAATA	720
AAAGCATTCT	TAAATGGACT	TGACAAATTT	AAGAGTTTAG	ATGTAAATAG	CTATAATAAA	780
AAAGCAAATT	ATATTAAAGG	TATGACAGAA	ATGGTTCTTG	ATCTAGTTGC	TCTATGGCCA	840
ACTTTCGATC	CAGATCATT	TCAAAAAGAA	GTAGAAATTG	AATTTACAAG	AACTATTTCT	900
TCTCCAATTT	ACCAACCTGT	ACCTAAAAAC	ATGCAAAATA	CCTCTAGCTC	TATGTACCT	960
AGCGATCTAT	TTCATATCA	AGGAGATCTT	GTAAAAATTAG	AATTTTCTAC	AAGAACGGAC	1020
AACGATGGTC	TTGCAAAAAT	TTTTACTGGT	ATTCGAAACA	CATTCTACAA	ATCGCCTAAT	1080
ACTCATGAAA	CATACCATGT	AGATTTTAGT	TATAATACCC	AATCTAGTGG	TAATATTTCA	1140
AGAGGCTCTT	CAAATCCGAT	TCCAATTGAT	CTTAATAATC	CCATTATTTT	AACTTGATTT	1200
AGAAATTCAT	TTTATAAGGC	AATAGCGGGA	TCTTCTGTTT	TAGTTAATTT	TAAAGATGGC	1260
ACTCAAGGGT	ATGCATTTGC	CCAAGCACCA	ACAGGAGGTG	CCTGGGACCA	TTCTTTTATT	1320
GAATCTGATG	GTGCCCCAGA	AGGGCATAAA	TTAAACTATA	TTTATACTTC	TCCAGGTGAT	1380
ACATTAAGAG	ATTTTCATCA	TGTATATACT	CTTATAAGTA	CTCCAATAT	AAATGAACCTA	1440
TCAACAGAAA	AAATCAAAGG	CTTTCCTGCG	GAAAAAGGAT	ATATCAAAAA	TCAAGGGATC	1500
ATGAAATATT	ACGGTAAACC	AGAATATATT	AATGGAGCTC	AACCAGTTAA	TCTGGAAAAC	1560
CAGCAAACAT	TAATATTGCA	ATTTTCATGCT	TCAAAAACAG	CTCAATATAC	CATTTCGTATA	1620
CGTTATGCCA	GTACCCAAGG	AACAAAAGGT	TATTTTCGTT	TAGATAATCA	GGAACCTGCAA	1680
ACGCTTAATA	TACCTACTTC	ACACAACGGT	TATGTAACCG	GTAATATTGG	TGAAAATTAT	1740
GATTTATATA	CAATAGGTTT	ATATACAATT	ACAGAAGGTA	ACCATACTCT	TCAAATCCAA	1800
CATAATGATA	AAAATGGAAT	GGTTTTAGAT	CGTATTGAAT	TTGTTCCCTAA	AGATTCACCTT	1860
CAAGATTAC	CTCAAGATT	ACCTCCAGAA	GTTACCGAAT	CAACAATTAT	TTTTGATAAA	1920
TCATCTCCAA	CTATATGGTC	TTCTAACAAA	CACCTCATATA	GCCATATACA	TTTAGAAGGA	1980
TCATATACAA	GTCAGGGAAG	TTATCCACAC	AATTTATTAA	TTAATTTATT	TCATCCTACA	2040
GACCCTAACA	GAAATCATAC	TATTCATGTT	AACAATGGTG	ATATGAATGT	TGATTATGGA	2100
AAAGATTCTG	TAGCCGATGG	GTTAAATTTT	AATAAAATAA	CTGCTACGAT	ACCAAGTGAT	2160
GCTTGGTATA	GCGGTACTAT	TACTTCTATG	CACCTATTTA	ATGATAATAA	TTTTAAAACA	2220
ATAACTCCTA	AATTTGAACT	TTCTAATGAA	TTAGAAAACA	TCACAACTCA	AGTAAATGCT	2280
TTATTGCGAT	CTAGTGCACA	AGATACTCTC	GCAAGTAATG	TAAGTGATTA	CTGGATTGAA	2340
CAGGTCGTTA	TGAAAGTCGA	TGCCTTATCA	GATGAAGTAT	TTGGAAAAGA	GAAAAAGCA	2400
TTACGTAAAT	TGGTAAATCA	AGCAAAACGT	CTCAGTAAAA	TACGAAATCT	TCTCATAGGT	2460
GGTAATTTTG	ACAATTTAGT	CGCTTGGTAT	ATGGGAAAAG	ATGTAGTAAA	AGAATCGGAT	2520
CATGAATTAT	TTAAAAGTGA	TCATGTCTTA	CTACCTCCCC	CAACATTCCA	TCCTTCTTAT	2580

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ATTTTCCAAA AGGTGGAAGA ATCAAAACTA AAACCAAATA CACGTTATAC TATTTCTGGT 2640
 TTTATCGCAC ATGGAGAAGA TGTAGAGCTT GTTGCTCTCTC GTTATGGGCA AGAAATACAA 2700
 AAAGTGATGC AAGTGCCATA TGAAGAAGCA CTTCTCTTA CATCTGAATC TAATCTAGT 2760
 TGTGTGTGTC CAAATTTAAA TATAAATGAA AACTAGCTG ATCCACATT CTTAGTTAT 2820
 AGCATCGATG TTGGTTCTCT GGAAATGGAA GCGAATCCTG GTATTGAATT TGGTCTCCGT 2880
 ATTGTCAAAC CAACAGGTAT GGCACGTGTA AGTAATTTAG AAATTCGAGA AGACCGTCCA 2940
 TTAACAGCAA AAGAAATTCG TCAAGTACAA CGTGCAGCAA GAGATTGGAA ACAAACACTAT 3000
 GAACAAGAAC GAACAGAGAT CACAGCTATA ATTCAACCTG TTCTTAATCA AATTAATGCG 3060
 TTATACGAAA ATGAAGATTG GAATGGTTCT ATTCGTTCAA ATGTTTCCTA TCATGATCTA 3120
 GAGCAAATTA TGCTTCCTAC TTTATTAATA ACTGAGGAAA TAAATTGTAA TTATGATCAT 3180
 CCAGCTTTTT TATTAAAAGT ATATCATTGG TTTATGACAG ATCGTATAGG AGAACATGGT 3240
 ACTATTTTAG CACGTTTCCA AGAAGCATT GATCGTGCAT ATACACAATT AGAAAGTCGT 3300
 AATCTCCTGC ATAACGGTCA TTTTACAAC GATACAGCGA ATTGGACAAT AGAAGGAGAT 3360
 GCCCATCATA CAATCTTAGA AGATGGTAGA CGTGTGTTAC GTTTACCAGA TTGGTCTTCT 3420
 AATGCAACTC AAACAATTGA AATTGAAGAT TTTGACTTAG ATCAAGAATA CCAATTGCTC 3480
 ATTCATGCAA AAGGAAAAGG TTCCATTACT TTACAACATG GAGAAGAAA CGAATATGTG 3540
 GAAACACATA CTCATCATA AAATGATTTT ATAACATCCC AAAATATTCC TTTCACTTTT 3600
 AAAGGAAATC AAATTGAAGT CCATATTACT TCAGAAGATG GAGAGTTTTT AATCGATCAC 3660
 ATTACAGTAA TAGAAGTTT TAAACAGAC ACAAATACAA ATATTATTGA AAATTCACCA 3720
 ATCAATACAA GTATGAATAG TAATGTAAGA GTAGATATAC CAAGAAGTCT C 3771

(2) INFORMATION FOR SEQ ID NO:6 (PS33F2):

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1257 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: *Bacillus thuringiensis*
 - (C) INDIVIDUAL ISOLATE: PS33F2
- (vii) IMMEDIATE SOURCE:
- (B) CLONE: E. coli NM522(pMYC 2316) B-18785
- (ix) FEATURE:
- (A) NAME/KEY: Protein
 - (B) LOCATION: 1..1257

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Thr Leu Asn Glu Val Tyr Pro Val Asn Tyr Asn Val Leu Ser
 1 5 10 15
 Ser Asp Ala Phe Gln Gln Leu Asp Thr Thr Gly Phe Lys Ser Lys Tyr
 20 25 30
 Asp Glu Met Ile Lys Ala Phe Glu Lys Lys Trp Lys Lys Gly Ala Lys
 35 40 45
 Gly Lys Asp Leu Leu Asp Val Ala Trp Thr Tyr Ile Thr Thr Gly Glu
 50 55 60
 Ile Asp Pro Leu Asn Val Ile Lys Gly Val Leu Ser Val Leu Thr Leu
 65 70 75 80
 Ile Pro Glu Val Gly Thr Val Ala Ser Ala Ala Ser Thr Ile Val Ser
 85 90 95

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Phe Ile Trp Pro Lys Ile Phe Gly Asp Lys Pro Asn Ala Lys Asn Ile
 100 105 110
 Phe Glu Glu Leu Lys Pro Gln Ile Glu Ala Leu Ile Gln Gln Asp Ile
 115 120 125
 Thr Asn Tyr Gln Asp Ala Ile Asn Gln Lys Lys Phe Asp Ser Leu Gln
 130 135 140
 Lys Thr Ile Asn Leu Tyr Thr Val Ala Ile Asp Asn Asn Asp Tyr Val
 145 150 155 160
 Thr Ala Lys Thr Gln Leu Glu Asn Leu Asn Ser Ile Leu Thr Ser Asp
 165 170 175
 Ile Ser Ile Phe Ile Pro Glu Gly Tyr Glu Thr Gly Gly Leu Pro Tyr
 180 185 190
 Tyr Ala Met Val Ala Asn Ala His Ile Leu Leu Leu Arg Asp Ala Ile
 195 200 205
 Val Asn Ala Glu Lys Leu Gly Phe Ser Asp Lys Glu Val Asp Thr His
 210 215 220
 Lys Lys Tyr Ile Lys Met Thr Ile His Asn His Thr Glu Ala Val Ile
 225 230 235 240
 Lys Ala Phe Leu Asn Gly Leu Asp Lys Phe Lys Ser Leu Asp Val Asn
 245 250 255
 Ser Tyr Asn Lys Lys Ala Asn Tyr Ile Lys Gly Met Thr Glu Met Val
 260 265 270
 Leu Asp Leu Val Ala Leu Trp Pro Thr Phe Asp Pro Asp His Tyr Gln
 275 280 285
 Lys Glu Val Glu Ile Glu Phe Thr Arg Thr Ile Ser Ser Pro Ile Tyr
 290 295 300
 Gln Pro Val Pro Lys Asn Met Gln Asn Thr Ser Ser Ser Ile Val Pro
 305 310 315 320
 Ser Asp Leu Phe His Tyr Gln Gly Asp Leu Val Lys Leu Glu Phe Ser
 325 330 335
 Thr Arg Thr Asp Asn Asp Gly Leu Ala Lys Ile Phe Thr Gly Ile Arg
 340 345 350
 Asn Thr Phe Tyr Lys Ser Pro Asn Thr His Glu Thr Tyr His Val Asp
 355 360 365
 Phe Ser Tyr Asn Thr Gln Ser Ser Gly Asn Ile Ser Arg Gly Ser Ser
 370 375 380
 Asn Pro Ile Pro Ile Asp Leu Asn Asn Pro Ile Ile Ser Thr Cys Ile
 385 390 395 400
 Arg Asn Ser Phe Tyr Lys Ala Ile Ala Gly Ser Ser Val Leu Val Asn
 405 410 415
 Phe Lys Asp Gly Thr Gln Gly Tyr Ala Phe Ala Gln Ala Pro Thr Gly
 420 425 430
 Gly Ala Trp Asp His Ser Phe Ile Glu Ser Asp Gly Ala Pro Glu Gly
 435 440 445
 His Lys Leu Asn Tyr Ile Tyr Thr Ser Pro Gly Asp Thr Leu Arg Asp
 450 455 460
 Phe Ile Asn Val Tyr Thr Leu Ile Ser Thr Pro Thr Ile Asn Glu Leu
 465 470 475 480
 Ser Thr Glu Lys Ile Lys Gly Phe Pro Ala Glu Lys Gly Tyr Ile Lys
 485 490 495
 Asn Gln Gly Ile Met Lys Tyr Tyr Gly Lys Pro Glu Tyr Ile Asn Gly
 500 505 510
 Ala Gln Pro Val Asn Leu Glu Asn Gln Gln Thr Leu Ile Phe Glu Phe
 515 520 525
 His Ala Ser Lys Thr Ala Gln Tyr Thr Ile Arg Ile Arg Tyr Ala Ser
 530 535 540
 Thr Gln Gly Thr Lys Gly Tyr Phe Arg Leu Asp Asn Gln Glu Leu Gln
 545 550 555 560

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Thr Leu Asn Ile Pro Thr Ser His Asn Gly Tyr Val Thr Gly Asn Ile
 565 570 575
 Gly Glu Asn Tyr Asp Leu Tyr Thr Ile Gly Ser Tyr Thr Ile Thr Glu
 580 585 590
 Gly Asn His Thr Leu Gln Ile Gln His Asn Asp Lys Asn Gly Met Val
 595 600 605
 Leu Asp Arg Ile Glu Phe Val Pro Lys Asp Ser Leu Gln Asp Ser Pro
 610 615 620
 Gln Asp Ser Pro Pro Glu Val His Glu Ser Thr Ile Ile Phe Asp Lys
 625 630 635 640
 Ser Ser Pro Thr Ile Trp Ser Ser Asn Lys His Ser Tyr Ser His Ile
 645 650 655
 His Leu Glu Gly Ser Tyr Thr Ser Gln Gly Ser Tyr Pro His Asn Leu
 660 665 670
 Leu Ile Asn Leu Phe His Pro Thr Asp Pro Asn Arg Asn His Thr Ile
 675 680 685
 His Val Asn Asn Gly Asp Met Asn Val Asp Tyr Gly Lys Asp Ser Val
 690 695 700
 Ala Asp Gly Leu Asn Phe Asn Lys Ile Thr Ala Thr Ile Pro Ser Asp
 705 710 715 720
 Ala Trp Tyr Ser Gly Thr Ile Thr Ser Met His Leu Phe Asn Asp Asn
 725 730 735
 Asn Phe Lys Thr Ile Thr Pro Lys Phe Glu Leu Ser Asn Glu Leu Glu
 740 745 750
 Asn Ile Thr Thr Gln Val Asn Ala Leu Phe Ala Ser Ser Ala Gln Asp
 755 760 765
 Thr Leu Ala Ser Asn Val Ser Asp Tyr Trp Ile Glu Gln Val Val Met
 770 775 780
 Lys Val Asp Ala Leu Ser Asp Glu Val Phe Gly Lys Glu Lys Lys Ala
 785 790 795 800
 Leu Arg Lys Leu Val Asn Gln Ala Lys Arg Leu Ser Lys Ile Arg Asn
 805 810 815
 Leu Leu Ile Gly Gly Asn Phe Asp Asn Leu Val Ala Trp Tyr Met Gly
 820 825 830
 Lys Asp Val Val Lys Glu Ser Asp His Glu Leu Phe Lys Ser Asp His
 835 840 845
 Val Leu Leu Pro Pro Pro Thr Phe His Pro Ser Tyr Ile Phe Gln Lys
 850 855 860
 Val Glu Glu Ser Lys Leu Lys Pro Asn Thr Arg Tyr Thr Ile Ser Gly
 865 870 875 880
 Phe Ile Ala His Gly Glu Asp Val Glu Leu Val Val Ser Arg Tyr Gly
 885 890 895
 Gln Glu Ile Gln Lys Val Met Gln Val Pro Tyr Glu Glu Ala Leu Pro
 900 905 910
 Leu Thr Ser Glu Ser Asn Ser Ser Cys Cys Val Pro Asn Leu Asn Ile
 915 920 925
 Asn Glu Thr Leu Ala Asp Pro His Phe Phe Ser Tyr Ser Ile Asp Val
 930 935 940
 Gly Ser Leu Glu Met Glu Ala Asn Pro Gly Ile Glu Phe Gly Leu Arg
 945 950 955 960
 Ile Val Lys Pro Thr Gly Met Ala Arg Val Ser Asn Leu Glu Ile Arg
 965 970 975
 Glu Asp Arg Pro Leu Thr Ala Lys Glu Ile Arg Gln Val Gln Arg Ala
 980 985 990
 Ala Arg Asp Trp Lys Gln Asn Tyr Glu Gln Glu Arg Thr Glu Ile Thr
 995 1000 1005
 Ala Ile Ile Gln Pro Val Leu Asn Gln Ile Asn Ala Leu Tyr Glu Asn
 1010 1015 1020
 Glu Asp Trp Asn Gly Ser Ile Arg Ser Asn Val Ser Tyr His Asp Leu

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1025	1030	1035	1040
Glu Gln Ile Met	Leu Pro Thr Leu Leu Lys Thr Glu Glu Ile Asn Cys		
	1045	1050	1055
Asn Tyr Asp His	Pro Ala Phe Leu Leu Lys Val Tyr His Trp Phe Met		
	1060	1065	1070
Thr Asp Arg Ile	Gly Glu His Gly Thr Ile Leu Ala Arg Phe Gln Glu		
	1075	1080	1085
Ala Leu Asp Arg	Ala Tyr Thr Gln Leu Glu Ser Arg Asn Leu Leu His		
	1090	1095	1100
Asn Gly His Phe	Thr Thr Asp Thr Ala Asn Trp Thr Ile Glu Gly Asp		
	1105	1110	1115
Ala His His Thr	Ile Leu Glu Asp Gly Arg Arg Val Leu Arg Leu Pro		
	1125	1130	1135
Asp Trp Ser Ser	Asn Ala Thr Gln Thr Ile Glu Ile Glu Asp Phe Asp		
	1140	1145	1150
Leu Asp Gln Glu	Tyr Gln Leu Leu Ile His Ala Lys Gly Lys Gly Ser		
	1155	1160	1165
Ile Thr Leu Gln	His Gly Glu Glu Asn Glu Tyr Val Glu Thr His Thr		
	1170	1175	1180
His His Thr Asn	Asp Phe Ile Thr Ser Gln Asn Ile Pro Phe Thr Phe		
	1185	1190	1195
Lys Gly Asn Gln	Ile Glu Val His Ile Thr Ser Glu Asp Gly Glu Phe		
	1205	1210	1215
Leu Ile Asp His	Ile Thr Val Ile Glu Val Ser Lys Thr Asp Thr Asn		
	1220	1225	1230
Thr Asn Ile Glu	Asn Ser Pro Ile Asn Thr Ser Met Asn Ser Asn		
	1235	1240	1245
Val Arg Val Asp	Ile Pro Arg Ser Leu		
	1250	1255	

(2) INFORMATION FOR SEQ ID NO:7 (PS52A1):

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1425 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: BACILLUS THURINGIENSIS
 - (C) INDIVIDUAL ISOLATE: PS52A1
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: E. coli NM522(pMYC 2321) B-18770
- (ix) FEATURE:
 - (A) NAME/KEY: mat peptide
 - (B) LOCATION: 1..1425
 - (D) OTHER INFORMATION: /product= "OPEN READING FRAME OF MATURE PROTEIN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGATTATTG ATAGTAAAC GACTTTACCT AGACATTCAC TTATTCATAC AATTAAATTA	60
AATTCTAATA AGAAATATGG TCCTGGTGAT ATGACTAATG GAAATCAATT TATTATTTCA	120
AAACAAGAAT GGGCTACGAT TGGAGCATAT ATTCAGACTG GATTAGGTTT ACCAGTAAAT	180
GAACAACAAT TAAGAACACA TGTTAATTTA AGTCAGGATA TATCAATACC TAGTGATTTT	240
TCTCAATTAT ATGATGTTA TTGTTCTGAT AAAACTTCAG CAGAATGGTG GAATAAAAAT	300
TTATATCCTT TAATTATTAA ATCTGCTAAT GATATTGCTT CATATGGTTT TAAAGTTGCT	360
GGTGATCCTT CTATTAAGAA AGATGGATAT TTTAAAAAAT TGCAAGATGA ATTAGATAAT	420

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ATTGTTGATA ATAATTC CGA TGATGATGCA ATAGCTAAAG CTATTAAAGA TTTTAAAGCG      480
CGATGTGGTA TTTTAATTAA AGAAGCTAAA CAATATGAAG AAGCTGCAAA AAATATTGTA      540
ACATCTTTAG ATCAATTTTT ACATGGTGAT CAGAAAAAAT TAGAAGGTGT TATCAATATT      600
CAAAAACGTT TAAAAGAAGT TCAAACAGCT CTTAATCAAG CCCATGGGGA AAGTAGTCCA      660
GCTCATAAAG AGTTATTAGA AAAAGTAAAA AATTTAAAAA CAACATTAGA AAGGACTATT      720
AAAGCTGAAC AAGATTTAGA GAAAAAAGTA GAATATAGTT TTCTATTAGG ACCATTGTTA      780
GGATTTGTTG TTTATGAAAT TCTTGAAAAT ACTGCTGTTC AGCATATAAA AAATCAAATT      840
GATGAGATAA AGAAACAATT AGATTCTGCT CAGCATGATT TGGATAGAGA TGTTAAATT      900
ATAGGAATGT TAAATAGTAT TAATACAGAT ATTGATAATT TATATAGTCA AGGACAAGAA      960
GCAATTAAAG TTTTCCAAAA GTTACAAGGT ATTTGGGCTA CTATTGGAGC TCAAATAGAA     1020
AATCTTAGAA CAACGTCGTT ACAAGAAGTT CAAGATTCTG ATGATGCTGA TGAGATACAA     1080
ATTGAAGTTG AGGACGCTTC TGATGCTTGG TTAGTTGTGG CTCAAGAAGC TCGTGATTTT     1140
ACACTAAATG CTTATTCAAC TAATAGTAGA CAAAATTTAC CGATTAATGT TATATCAGAT     1200
TCATGTAATT GTTCAACAAC AAATATGACA TCAAATCAAT ACAGTAATCC AACAACAAAT     1260
ATGACATCAA ATCAATATAT GATTTCACAT GAATATACAA GTTTACCAA TAATTTTATG     1320
TTATCAAGAA ATAGTAATTT AGAATATAAA TGTCTGAAA ATAATTTTAT GATATATTGG     1380
TATAATAATT CGGATTGGTA TAATAATTCG GATTGGTATA ATAAT                                1425

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(2) INFORMATION FOR SEQ ID NO:8 (PS52A1):

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 475 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: BACILLUS THURINGIENSIS
 - (C) INDIVIDUAL ISOLATE: PS52A1
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: E. coli NM522(pMYC 2321) B-18770
- (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..475
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:


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Met Ile Ile Asp Ser Lys Thr Thr Leu Pro Arg His Ser Leu Ile His
 1           5           10
Thr Ile Lys Leu Asn Ser Asn Lys Lys Tyr Gly Pro Gly Asp Met Thr
          20           25           30
Asn Gly Asn Gln Phe Ile Ile Ser Lys Gln Glu Trp Ala Thr Ile Gly
          35           40           45
Ala Tyr Ile Gln Thr Gly Leu Gly Leu Pro Val Asn Glu Gln Gln Leu
          50           55           60
Arg Thr His Val Asn Leu Ser Gln Asp Ile Ser Ile Pro Ser Asp Phe
          65           70           75           80
Ser Gln Leu Tyr Asp Val Tyr Cys Ser Asp Lys Thr Ser Ala Glu Trp
          85           90           95
Trp Asn Lys Asn Leu Tyr Pro Leu Ile Ile Lys Ser Ala Asn Asp Ile
          100          105          110
Ala Ser Tyr Gly Phe Lys Val Ala Gly Asp Pro Ser Ile Lys Lys Asp
          115          120          125

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Gly Tyr Phe Lys Lys Leu Gln Asp Glu Leu Asp Asn Ile Val Asp Asn
 130 135 140
 Asn Ser Asp Asp Asp Ala Ile Ala Lys Ala Ile Lys Asp Phe Lys Ala
 145 150 155 160
 Arg Cys Gly Ile Leu Ile Lys Glu Ala Lys Gln Tyr Glu Glu Ala Ala
 165 170 175
 Lys Asn Ile Val Thr Ser Leu Asp Gln Phe Leu His Gly Asp Gln Lys
 180 185 190
 Lys Leu Glu Gly Val Ile Asn Ile Gln Lys Arg Leu Lys Glu Val Gln
 195 200 205
 Thr Ala Leu Asn Gln Ala His Gly Glu Ser Ser Pro Ala His Lys Glu
 210 215 220
 Leu Leu Glu Lys Val Lys Asn Leu Lys Thr Thr Leu Glu Arg Thr Ile
 225 230 235 240
 Lys Ala Glu Gln Asp Leu Glu Lys Lys Val Glu Tyr Ser Phe Leu Leu
 245 250 255
 Gly Pro Leu Leu Gly Phe Val Val Tyr Glu Ile Leu Glu Asn Thr Ala
 260 265 270
 Val Gln His Ile Lys Asn Gln Ile Asp Glu Ile Lys Lys Gln Leu Asp
 275 280 285
 Ser Ala Gln His Asp Leu Asp Arg Asp Val Lys Ile Ile Gly Met Leu
 290 295 300
 Asn Ser Ile Asn Thr Asp Ile Asp Asn Leu Tyr Ser Gln Gly Gln Glu
 305 310 315 320
 Ala Ile Lys Val Phe Gln Lys Leu Gln Gly Ile Trp Ala Thr Ile Gly
 325 330 335
 Ala Gln Ile Glu Asn Leu Arg Thr Thr Ser Leu Gln Glu Val Gln Asp
 340 345 350
 Ser Asp Asp Ala Asp Glu Ile Gln Ile Glu Leu Glu Asp Ala Ser Asp
 355 360 365
 Ala Trp Leu Val Val Ala Gln Glu Ala Arg Asp Phe Thr Leu Asn Ala
 370 375 380
 Tyr Ser Thr Asn Ser Arg Gln Asn Leu Pro Ile Asn Val Ile Ser Asp
 385 390 395 400
 Ser Cys Asn Cys Ser Thr Thr Asn Met Thr Ser Asn Gln Tyr Ser Asn
 405 410 415
 Pro Thr Thr Asn Met Thr Ser Asn Gln Tyr Met Ile Ser His Glu Tyr
 420 425 430
 Thr Ser Leu Pro Asn Asn Phe Met Leu Ser Arg Asn Ser Asn Leu Glu
 435 440 445
 Tyr Lys Cys Pro Glu Asn Asn Phe Met Ile Tyr Trp Tyr Asn Asn Ser
 450 455 460
 Asp Trp Tyr Asn Asn Ser Asp Trp Tyr Asn Asn
 465 470 475

(2) INFORMATION FOR SEQ ID NO:9 (PS69D1):

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1185 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: BACILLUS THURINGIENSIS
- (C) INDIVIDUAL ISOLATE: PS69D1

(vii) IMMEDIATE SOURCE:

- (B) CLONE: E. coli NM522(pMYC2317) NRRL B-18816

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- (ix) FEATURE:
 (A) NAME/KEY: mat peptide
 (B) LOCATION: 1..1185

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGATTTTAG GGAATGGAAA GACTTTACCA AAGCATATAA GATTAGCTCA TATTTTGTGCA	60
ACACAGAATT CTTCAGCTAA GAAAGACAAT CCTCTTGGAC CAGAGGGGAT GGTTACTAAA	120
GACGGTTTAA TAATCTCTAA GGAAGAATGG GCATTTGTGC AGGCCTATGT GACTACAGGC	180
ACTGGTTTAC CTATCAATGA CGATGAGATG CGTAGACATG TTGGGTACC ATCAGCGATT	240
CAAATTCCTG ATGATTTTAA TCAATTATAT AAGGTTTATA ATGAAGATAA ACATTTATGC	300
AGTTGGTGGA ATGGTTTCTT GTTTCATTA GTTCTTAAAA CAGCTAATGA TATTTCCGCT	360
TACGGATTTA AATGTGCTGG AAAGGGTGCC ACTAAAGGAT ATTATGAGGT CATGCAAGAC	420
GATGTAGAAA ATATTTTCAGA TAATGGTTAT GATAAGTTG CACAAGAAAA AGCACATAAG	480
GATCTGCAGG CGCGTTGTAA AATCCTTATT AAGGAGGCTG ATCAATATAA AGCTGCAGCG	540
GATGATGTTT CAAAACATTT AAACACATTT CTAAAGGCG GTCAAGATTC AGATGGCAAT	600
GATGTTATTG GCGTAGAGGC TGTCAAGTA CAACTAGCAC AAGTAAAGA TAATCTTGAT	660
GGCCTATATG GCGACAAAAG CCCAAGACAT GAAGAGTTAC TAAAGAAAGT AGACGACCTG	720
AAAAAGAGT TGGAAGCTGC TATTAAAGCA GAGAATGAAT TAGAAAAGAA AGTGAAAATG	780
AGTTTGTCTT TAGGACCATT ACTTGATTT GTTGATATG AAATCTTAGA GCTAACTGCG	840
GTCAAAAGTA TACACAAGAA AGTTGAGGCA CTACAAGCCG AGCTTGACAC TGCTAATGAT	900
GAAGTCGACA GAGATGTAAA AATCTTAGGA ATGATGAATA GCATTGACAC TGATATTGAC	960
AACATGTTAG AGCAAGGTGA GCAAGCTCTT GTTGATTTA GAAAAATTGC AGGCATTTGG	1020
AGTGTATATA GTCTTAATAT CGGCAATCTT CGAGAAACAT CTTTAAAAGA GATAGAAGAA	1080
GAAAATGATG ACGATGCACT GTATATTGAG CTTGGTGATG CCGCTGGTCA ATGGAAAGAG	1140
ATAGCCGAGG AGGCACAATC CTTGTACTA AATGCTTATA CTCCT	1185

(2) INFORMATION FOR SEQ ID NO:10 (PS69D1):

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 395 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: BACILLUS THURINGIENSIS
 (C) INDIVIDUAL ISOLATE: PS69D1
- (vii) IMMEDIATE SOURCE:
 (B) CLONE: E. coli NM522(pMYC2317) NRRL B-18816
- (ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..395

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met	Ile	Leu	Gly	Asn	Gly	Lys	Thr	Leu	Pro	Lys	His	Ile	Arg	Leu	Ala
1				5				10						15	
His	Ile	Phe	Ala	Thr	Gln	Asn	Ser	Ser	Ala	Lys	Lys	Asp	Asn	Pro	Leu
		20					25						30		
Gly	Pro	Glu	Gly	Met	Val	Thr	Lys	Asp	Gly	Phe	Ile	Ile	Ser	Lys	Glu
		35				40					45				
Glu	Trp	Ala	Phe	Val	Gln	Ala	Tyr	Val	Thr	Thr	Gly	Thr	Gly	Leu	Pro
50					55						60				

61

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Ile Asn Asp Asp Glu Met Arg Arg His Val Gly Leu Pro Ser Arg Ile
65      70      75      80
Gln Ile Pro Asp Asp Phe Asn Gln Leu Tyr Lys Val Tyr Asn Glu Asp
      85      90      95
Lys His Leu Cys Ser Trp Trp Asn Gly Phe Leu Phe Pro Leu Val Leu
      100      105      110
Lys Thr Ala Asn Asp Ile Ser Ala Tyr Gly Phe Lys Cys Ala Gly Lys
      115      120      125
Gly Ala Thr Lys Gly Tyr Tyr Glu Val Met Gln Asp Asp Val Glu Asn
      130      135      140
Ile Ser Asp Asn Gly Tyr Asp Lys Val Ala Gln Glu Lys Ala His Lys
145      150      155      160
Asp Leu Gln Ala Arg Cys Lys Ile Leu Ile Lys Glu Ala Asp Gln Tyr
      165      170      175
Lys Ala Ala Ala Asp Asp Val Ser Lys His Leu Asn Thr Phe Leu Lys
      180      185      190
Gly Gly Gln Asp Ser Asp Gly Asn Asp Val Ile Gly Val Glu Ala Val
      195      200      205
Gln Val Gln Leu Ala Gln Val Lys Asp Asn Leu Asp Gly Leu Tyr Gly
      210      215      220
Asp Lys Ser Pro Arg His Glu Glu Leu Leu Lys Lys Val Asp Asp Leu
225      230      235      240
Lys Lys Glu Leu Glu Ala Ala Ile Lys Ala Glu Asn Glu Leu Glu Lys
      245      250      255
Lys Val Lys Met Ser Phe Ala Leu Gly Pro Leu Leu Gly Phe Val Val
      260      265      270
Tyr Glu Ile Leu Glu Leu Thr Ala Val Lys Ser Ile His Lys Lys Val
      275      280      285
Glu Ala Leu Gln Ala Glu Leu Asp Thr Ala Asn Asp Glu Leu Asp Arg
      290      295      300
Asp Val Lys Ile Leu Gly Met Met Asn Ser Ile Asp Thr Asp Ile Asp
305      310      315      320
Asn Met Leu Glu Gln Gly Glu Gln Ala Leu Val Val Phe Arg Lys Ile
      325      330      335
Ala Gly Ile Trp Ser Val Ile Ser Leu Asn Ile Gly Asn Leu Arg Glu
      340      345      350
Thr Ser Leu Lys Glu Ile Glu Glu Glu Asn Asp Asp Asp Ala Leu Tyr
      355      360      365
Ile Glu Leu Gly Asp Ala Ala Gly Gln Trp Lys Glu Ile Ala Glu Glu
      370      375      380
Ala Gln Ser Phe Val Leu Asn Ala Tyr Thr Pro
385      390      395

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(2) INFORMATION FOR SEQ ID NO:11

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2412 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Bacillus thuringiensis*
- (C) INDIVIDUAL ISOLATE: PS63B

(vii) IMMEDIATE SOURCE:

- (B) CLONE: E. coli NM522(pMYC 1642) NRRL B-18961

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGACTTGTC AATTACAAGC GCAACCACTT ATTCCTATA ACGTACTAGC AGGAGTTCCA

60

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ACTAGTAATA CAGGTAGTCC AATCGGCAAT GCAGGTAATC AATTTGATCA GTTTGAGCAA	120
ACCGTTAAAG AGCTCAAGGA AGCATGGGAA GCGTTCCAAA AAAACGGAAG TTCTCATT	180
GCAGCTCTTG AAAAGGGATT TGATGCAGCA ATCGGAGGAG GATCCTTTGA TTATTTAGGT	240
TTAGTTCAAG CCGGCCTAGG ATTAGTTGGT ACGCTAGGCG CCGCAATCCC TGGTGTTC	300
GTGGCAGTGC CTCTTATTAG CATGCTTGTT GGTGTTTTTT GGCCAAAGGG CACAAACAAC	360
CAAGAAAACC TTATTACAGT TATTGATAAG GAAGTTCAGA GAATACTAGA TGAAAAGCTA	420
TCTGATCAGT TAATAAGAA ATTGAACGCA GATTAAATG CTTTTACGGA CCTAGTAACT	480
CGTTTGGAAG AAGTAATAAT AGATGCAACT TTCGAGAATC ACAAGCCTGT ACTACAAGTA	540
AGTAAATCAA ATTATATGAA AGTGGATTCA GCATATTTCT CAACAGGAGG TATTCTTACT	600
CTTGGCATGA GTGATTTTCT TACTGATACC TATTCAAAGC TTACCTTCCC ATTATATGTA	660
CTAGGCGCAA CTATGAAACT TTCAGCATAT CATAGTTATA TACAATTCGG AAATACATGG	720
CTTAATAAAG TTTATGATTT ATCATCAGAT GAGGGAAAA CAATGTCGCA GGCTTTAGCA	780
CGAGCTAAAC AGCATATGCG CCAAGACATA GCATTTTATA CAAGCCAAGC TTAAACATG	840
TTTACTGGGA ATCTCCCTTC ATTATCATCT AATAAATATG CAATTAATGA CTATAATGTA	900
TACACTCGAG CAATGGTATT GAATGGCTTA GATATAGTAG CAACATGGCC TACCCTATAT	960
CCAGATGACT ATTCGTCTCA GATAAACTG GAGAAAACAC GCGTGATCTT TTCAGATATG	1020
GTGCGGCAA GTGAGAGTAG AGATGGCAGC GTAACGATTA AAAATATTTT TGACAATACA	1080
GATTCACATC AACATGGATC CATAGGTCTC AATTCAATCT CTTATTTCCC AGATGAGTTA	1140
CAGAAAGCAC AACTTCGCAT GTATGATTAT AATCACAAC CTTATTGTAC GGACTGTTTC	1200
TGCTGGCCGT ATGGAGTGAT TTTAACTAT AACAAGAATA CCTTTAGATA TGGCGATAAT	1260
GATCCAGGTC TTTCAGGAGA CGTTCAACTC CCAGCACCTA TGAGTGTAGT TAATGCCCAA	1320
ACTCAAACAG CCCAATATAC AGATGGAGAA AACATATGGA CAGATACTGG CCGCAGTTGG	1380
CTTTGTACTC TACGTGGCTA CTGTACTACA AACTGTTTTT CAGGAAGAGG TTGTTATAAT	1440
AATAGTACTG GATATGGAGA AAGTTGCAAT CAATCACTTC CAGGTCAAAA AATACATGCA	1500
CTATATCCTT TTACACAAAC AAATGTGCTG GGACAATCAG GCAAACCTAGG ATTGCTAGCA	1560
AGTCATATTC CATATGACCT AAGTCCGAAC AATACGATTG GTGACAAAGA TACAGATTCT	1620
ACGAATATTG TCGCAAAAGG AATTCCAGTG GAAAAAGGGT ATGCATCCAG TGGACAAAAA	1680
GTGAAATTA TACGAGAGTG GATAAATGGT GCGAATGTAG TTCAATTATC TCCAGGCCAA	1740
TCTTGGGGAA TGGATTTTAC CAATAGCACA GGTGGTCAAT ATATGGTCCG CTGTCGATAT	1800
GCAAGTACAA ACGATACTCC AATCTTTTTT AATTTAGTGT ATGACGGGGG ATCGAATCCT	1860
ATTTATAACC AGATGACATT CCTGTCTACA AAAGAGACTC CAGCTCACGA TTCAGTAGAT	1920
AACAAGATAC TAGGCATAAA AGGAATAAAT GGAAATTATT CACTCATGAA TGTAAAAGAT	1980
TCGTGCGAAC TTCCATCTGG GAAATTTTCAT GTTTTTTTCA CAAATAATGG ATCATCTGCT	2040
ATTTATTTAG ATCGACTTGA GTTTGTTTCT TTAGATCAAC CAGCAGCGCC AACACAGTCA	2100
ACACAACCAA TTAATTATCC TATCACAAGT AGGTTACCTC ATCGTTCCGG AGAACCACCT	2160
GCAATAATAT GGGAGAAATC AGGGAATGTT CGCGGGAATC AACTAACTAT ATCGGCACAA	2220
GGTGTTCAG AAAATTCCCA AATATATCTT TCGGTGGGTG GCGATCGCCA AATTTTAGAC	2280
CGTAGCAACG GATTTAAATT AGTTAATTAC TCACCTACTT ATCTTTTCAC TAACATTCAG	2340
GCTAGCTCGT CAAATTTAGT AGATATTACA AGTGGTACCA TCACTGGCCA AGTACAAGTA	2400
TCTAATCTAT AA	2412

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 803 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

63

(ii) MOLECULE TYPE: protein
 (iii) HYPOTHETICAL: YES
 (iv) ANTI-SENSE: NO
 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Bacillus thuringiensis*
 (C) INDIVIDUAL ISOLATE: PS63B
 (vii) IMMEDIATE SOURCE:
 (B) CLONE: *E. coli* NM522(pMYC 1642) NRRL B-18961
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Thr Cys Gln Leu Gln Ala Gln Pro Leu Ile Pro Tyr Asn Val Leu
 1 5 10 15
 Ala Gly Val Pro Thr Ser Asn Thr Gly Ser Pro Ile Gly Asn Ala Gly
 20 25 30
 Asn Gln Phe Asp Gln Phe Glu Gln Thr Val Lys Glu Leu Lys Glu Ala
 35 40 45
 Trp Glu Ala Phe Gln Lys Asn Gly Ser Phe Ser Leu Ala Ala Leu Glu
 50 55 60
 Lys Gly Phe Asp Ala Ala Ile Gly Gly Gly Ser Phe Asp Tyr Leu Gly
 65 70 75 80
 Leu Val Gln Ala Gly Leu Gly Leu Val Gly Thr Leu Gly Ala Ala Ile
 85 90 95
 Pro Gly Val Ser Val Ala Val Pro Leu Ile Ser Met Leu Val Gly Val
 100 105 110
 Phe Trp Pro Lys Gly Thr Asn Asn Gln Glu Asn Leu Ile Thr Val Ile
 115 120 125
 Asp Lys Glu Val Gln Arg Ile Leu Asp Glu Lys Leu Ser Asp Gln Leu
 130 135 140
 Ile Lys Lys Leu Asn Ala Asp Leu Asn Ala Phe Thr Asp Leu Val Thr
 145 150 155 160
 Arg Leu Glu Glu Val Ile Ile Asp Ala Thr Phe Glu Asn His Lys Pro
 165 170 175
 Val Leu Gln Val Ser Lys Ser Asn Tyr Met Lys Val Asp Ser Ala Tyr
 180 185 190
 Phe Ser Thr Gly Gly Ile Leu Thr Leu Gly Met Ser Asp Phe Leu Thr
 195 200 205
 Asp Thr Tyr Ser Lys Leu Thr Phe Pro Leu Tyr Val Leu Gly Ala Thr
 210 215 220
 Met Lys Leu Ser Ala Tyr His Ser Tyr Ile Gln Phe Gly Asn Thr Trp
 225 230 235 240
 Leu Asn Lys Val Tyr Asp Leu Ser Ser Asp Glu Gly Lys Thr Met Ser
 245 250 255
 Gln Ala Leu Ala Arg Ala Lys Gln His Met Arg Gln Asp Ile Ala Phe
 260 265 270
 Tyr Thr Ser Gln Ala Leu Asn Met Phe Thr Gly Asn Leu Pro Ser Leu
 275 280 285
 Ser Ser Asn Lys Tyr Ala Ile Asn Asp Tyr Asn Val Tyr Thr Arg Ala
 290 295 300
 Met Val Leu Asn Gly Leu Asp Ile Val Ala Thr Trp Pro Thr Leu Tyr
 305 310 315 320
 Pro Asp Asp Tyr Ser Ser Gln Ile Lys Leu Glu Lys Thr Arg Val Ile
 325 330 335
 Phe Ser Asp Met Val Gly Gln Ser Glu Ser Arg Asp Gly Ser Val Thr
 340 345 350
 Ile Lys Asn Ile Phe Asp Asn Thr Asp Ser His Gln His Gly Ser Ile
 355 360 365
 Gly Leu Asn Ser Ile Ser Tyr Phe Pro Asp Glu Leu Gln Lys Ala Gln
 370 375 380

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Leu Arg Met Tyr Asp Tyr Asn His Lys Pro Tyr Cys Thr Asp Cys Phe
 385 390 395 400
 Cys Trp Pro Tyr Gly Val Ile Leu Asn Tyr Asn Lys Asn Thr Phe Arg
 405 410 415
 Tyr Gly Asp Asn Asp Pro Gly Leu Ser Gly Asp Val Gln Leu Pro Ala
 420 425 430
 Pro Met Ser Val Val Asn Ala Gln Thr Gln Thr Ala Gln Tyr Thr Asp
 435 440 445
 Gly Glu Asn Ile Trp Thr Asp Thr Gly Arg Ser Trp Leu Cys Thr Leu
 450 455 460
 Arg Gly Tyr Cys Thr Thr Asn Cys Phe Pro Gly Arg Gly Cys Tyr Asn
 465 470 475 480
 Asn Ser Thr Gly Tyr Gly Glu Ser Cys Asn Gln Ser Leu Pro Gly Gln
 485 490 495
 Lys Ile His Ala Leu Tyr Pro Phe Thr Gln Thr Asn Val Leu Gly Gln
 500 505 510
 Ser Gly Lys Leu Gly Leu Leu Ala Ser His Ile Pro Tyr Asp Leu Ser
 515 520 525
 Pro Asn Asn Thr Ile Gly Asp Lys Asp Thr Asp Ser Thr Asn Ile Val
 530 535 540
 Ala Lys Gly Ile Pro Val Glu Lys Gly Tyr Ala Ser Ser Gly Gln Lys
 545 550 555 560
 Val Glu Ile Ile Arg Glu Trp Ile Asn Gly Ala Asn Val Val Gln Leu
 565 570 575
 Ser Pro Gly Gln Ser Trp Gly Met Asp Phe Thr Asn Ser Thr Gly Gly
 580 585 590
 Gln Tyr Met Val Arg Cys Arg Tyr Ala Ser Thr Asn Asp Thr Pro Ile
 595 600 605
 Phe Phe Asn Leu Val Tyr Asp Gly Gly Ser Asn Pro Ile Tyr Asn Gln
 610 615 620
 Met Thr Phe Pro Ala Thr Lys Glu Thr Pro Ala His Asp Ser Val Asp
 625 630 635 640
 Asn Lys Ile Leu Gly Ile Lys Gly Ile Asn Gly Asn Tyr Ser Leu Met
 645 650 655
 Asn Val Lys Asp Ser Val Glu Leu Pro Ser Gly Lys Phe His Val Phe
 660 665 670
 Phe Thr Asn Asn Gly Ser Ser Ala Ile Tyr Leu Asp Arg Leu Glu Phe
 675 680 685
 Val Pro Leu Asp Gln Pro Ala Ala Pro Thr Gln Ser Thr Gln Pro Ile
 690 695 700
 Asn Tyr Pro Ile Thr Ser Arg Leu Pro His Arg Ser Gly Glu Pro Pro
 705 710 715 720
 Ala Ile Ile Trp Glu Lys Ser Gly Asn Val Arg Gly Asn Gln Leu Thr
 725 730 735
 Ile Ser Ala Gln Gly Val Pro Glu Asn Ser Gln Ile Tyr Leu Ser Val
 740 745 750
 Gly Gly Asp Arg Gln Ile Leu Asp Arg Ser Asn Gly Phe Lys Leu Val
 755 760 765
 Asn Tyr Ser Pro Thr Tyr Ser Phe Thr Asn Ile Gln Ala Ser Ser Ser
 770 775 780
 Asn Leu Val Asp Ile Thr Ser Gly Thr Ile Thr Gly Gln Val Gln Val
 785 790 795 800
 Ser Asn Leu

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 8 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

65

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Arg Glu Trp Ile Asn Gly Ala Asn
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AGATRKWTW AATGGWGCKM AW

22

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Pro Thr Phe Asp Pro Asp Leu Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCNACYTTTK ATCCAGATSW YTAT

24

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala Ile Leu Asn Glu Leu Tyr Pro Ser Val Pro Tyr Asn Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ala Ile Leu Asn Glu Leu Tyr Pro Ser Val Pro Tyr Asn Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 17 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Ile Ile Asp Ser Lys Thr Thr Leu Pro Arg His Ser Leu Ile Asn
 1 5 10 15
 Thr

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gln Leu Gln Ala Gln Pro Leu Ile Pro Tyr Asn Val Leu Ala
 1 5 10

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Ile Leu Gly Asn Gly Lys Thr Leu Pro Lys His Ile Arg Leu Ala
 1 5 10 15
 His Ile Phe Ala Thr Gln Asn Ser
 20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ala Thr Leu Asn Glu Val Tyr Pro Val Asn
 1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Val Gln Arg Ile Leu Asp Glu Lys Leu Ser Phe Gln Leu Ile Lys
 1 5 10 15

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

67

(ii) MOLECULE TYPE: DNA (synthetic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
GCAATTTTAA ATGAATTATA TCC

23

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 56 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (synthetic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
ATGATTATTG ATTCTAAAAC AACATTACCA AGACATTCTT TAATWAATAC WATWAA

56

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 38 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (synthetic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
AAACATATTA GATTAGCACA TATTTTGTGCA ACACAAAA

38

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (synthetic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
CAAYTACAAG CWCAACC

17

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (synthetic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
TTCATCTAAA ATTCTTTGWA C

21

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 8 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
Leu Asp Arg Ile Gln Phe Ile Pro
1 5

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 bases
 (B) TYPE: nucleic acid

68

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AGGAACAAAY TCAAKWCGRT CTA

23

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Tyr Ile Asp Lys Ile Glu Phe Ile Pro
1 5

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 bases
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TGGAATAAAT TCAATTYKRT CWA

23

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 bases
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GCWACWTTAA ATGAAGTWA T

21

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 bases
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AATGAAGTWT ATCCWGTWAA T

21

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 38 bases
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GCAAGCGGCC GCTTATGGAA TAAATTCAAT TYKRTCWA

38

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 28 bases
 - (B) TYPE: nucleic acid

69

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TGATTTTWT CAATTATATR AKGTTTAT

28

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

AAGAGTTAYT ARARAAAGTA

20

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TTAGGACCAT TRYTWGGATT TGTGTWTAT GAAAT

35

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GAYAGAGATG TWAAAATYWT AGGAATG

27

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TTMTTAAAWC WGCTAATGAT ATT

23

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Claims

1 1. A substantially pure toxin protein which is toxic to nematodes and which has at least
2 one characteristic selected from the group consisting of:

- 3 (a) the amino acid sequence of said toxin conforms to either Generic Formula I or
4 Generic Formula II;
5 (b) the amino acid sequence of said toxin is at least 50% homologous with the amino
6 acid sequence of a protein selected from the group consisting of toxins 17, 33F2,
7 52A1, 63B, and 69D1;
8 (c) the amino acid sequence of said toxin has an alignment value of at least 100 with
9 either toxin 17 or toxin 52A1;
10 (d) the DNA which codes for said toxin hybridizes with DNA which codes for all or
11 part of a protein selected from the group consisting of toxins 17, 33F2, 52A1,
12 63B, and 69D1;
13 (e) the DNA which codes for said toxin hybridizes with a probe selected from the
14 group consisting of SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 30, SEQ ID
15 NO. 36, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, and SEQ ID NO.
16 40;
17 (f) a portion of the nucleotide sequence coding for said toxin can be amplified from
18 total cellular DNA from a *Bacillus thuringiensis* strain using polymerase chain
19 reaction with a reverse primer selected from the group consisting of SEQ ID NO.
20 30, SEQ ID NO. 32, and the complement of SEQ ID NO. 14; and a forward
21 primer selected from the group consisting of SEQ ID NO. 14, SEQ ID NO. 16,
22 SEQ ID NO. 5 (Probe B), SEQ ID NO. 24, and SEQ ID NO. 27; and
23 (g) a portion of the nucleotide sequence coding for said toxin can be amplified from
24 a *Bacillus thuringiensis* strain using polymerase chain reaction with
25 (i) a forward primer which is either SEQ ID NO. 36 or SEQ ID NO. 40
26 and a reverse primer which is complementary to either SEQ ID NO. 37,
27 SEQ ID NO. 38, or SEQ ID NO. 39;
28 (ii) a forward primer which SEQ ID NO. 37 and a reverse primer which is
29 complementary to either SEQ ID NO. 38 or SEQ ID NO. 39;
30 (h) said toxin is immunoreactive with an antibody which immunoreacts with a
31 protein selected from the group consisting of toxins 17, 33F2, 52A1, 63B, and
32 69D1.

1 2. The nematode toxin, according to claim 1, wherein said toxin has an amino acid
2 sequence according to Generic Formula I.

1 3. The nematode toxin, according to claim 2, wherein said toxin has a molecular weight
2 between about 65 kDa and about 155 kDa.

1 4. The nematode toxin, according to claim 1, wherein said toxin has an amino acid
2 sequence according to Generic Formula II.

1 5. The nematode toxin, according to claim 4, wherein said toxin has a molecular weight
2 between about 45 kDa and about 65 kDa.

1 6. The nematode toxin, according to claim 1, wherein said toxin has an alignment value
2 of at least 100 with toxin 17.

1 7. The nematode toxin, according to claim 1, wherein said toxin has an alignment value
2 of at least 100 with toxin 52A1.

1 8. The nematode toxin, according to claim 1, wherein the DNA coding for said toxin
2 hybridizes with DNA which codes for all or part of a protein selected from the group consisting
3 of toxins 17, 33F2, 52A1, 63B, and 69D1.

1 9. The nematode toxin, according to claim 1, wherein the DNA coding for said toxin
2 hybridizes with a probe selected from the group consisting of SEQ ID NO. 14 and SEQ ID NO.
3 16.

1 10. The nematode toxin, according to claim 1, wherein a portion of the nucleotide
2 sequence coding for said toxin can be amplified from total cellular DNA from a *Bacillus*
3 *thuringiensis* strain using polymerase chain reaction with a reverse primer selected from the group
4 consisting of SEQ ID NO. 30, SEQ ID NO. 32, and the complement of SEQ ID NO. 14; and a
5 forward primer selected from the group consisting of SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID
6 NO. 5 (Probe B), SEQ ID NO. 24, and SEQ ID NO. 27.

1 11. The nematode toxin, according to claim 10, wherein said reverse primer is SEQ ID
2 NO. 32 or SEQ ID NO. 30 and

3 (a) the forward primer is SEQ ID NO. 14 and the polymerase chain reaction
4 fragment is approximately 330 to 600 bp;

5 (b) the forward primer is SEQ ID NO. 16 and the polymerase chain reaction
6 fragment is approximately 1000 to 1400 bp; or

- 7 (c) the forward primer is either SEQ ID NO. 5 (Probe B), SEQ ID NO. 24, or SEQ
8 ID NO. 27 and the polymerase chain reaction fragment is 1800 to 2100 bp.

1 12. The nematode toxin, according to claim 10, wherein said reverse primer is a
2 complement of SEQ ID NO. 14 and

- 3 (a) the forward primer is SEQ ID NO. 16 and the polymerase chain reaction
4 fragment is approximately 650 to 1000 bp; or
5 (b) the forward primer is SEQ ID NO. 5 (Probe B), SEQ ID NO. 24, or SEQ ID
6 NO. 27 and the polymerase chain reaction fragment is approximately 1000 to
7 1400 bp.

1 13. The nematode toxin, according to claim 1, wherein a portion of the nucleotide
2 sequence coding for said toxin can be amplified from a *Bacillus thuringiensis* strain using
3 polymerase chain reaction with

- 4 (a) a forward primer which is either SEQ ID NO. 36 or SEQ ID NO. 40 and a
5 reverse primer which is complementary to either SEQ ID NO. 37, SEQ ID NO.
6 38, or SEQ ID NO. 39; or
7 (b) a forward primer which is SEQ ID NO. 37 and a reverse primer which is
8 complementary to either SEQ ID NO. 38 or SEQ ID NO. 39.

1 14. The nematode toxin, according to claim 13, wherein said forward primer is SEQ ID
2 NO. 36 and

- 3 (a) said reverse primer is complementary to SEQ ID NO. 37 and produces a
4 polymerase chain reaction fragment of about 440 bp;
5 (b) said reverse primer is complementary to SEQ ID NO. 38 and produces a
6 polymerase chain reaction fragment of about 540 bp; or
7 (c) said reverse primer is complementary to SEQ ID NO. 39 and produces a
8 polymerase chain reaction fragment of about 650 bp.

1 15. The nematode toxin, according to claim 13, wherein said forward primer is SEQ ID
2 NO. 40 which, when used with reverse primers which are complementary to SEQ ID NOS. 37, 38,
3 or 39, yields polymerase chain reaction fragments of about 360, 460, and 570 bp, respectively.

1 16. The nematode toxin, according to claim 13, wherein said forward primer is SEQ ID
2 NO. 37 which, when used with reverse primers complementary to SEQ ID NOS. 38 or 39, yields
3 polymerase chain reaction fragments of about 100 and 215 bp, respectively.

1 17. The nematode toxin, according to claim 1, wherein said toxin is immunoreactive with
2 an antibody which is immunoreactive with a protein selected from the group consisting of toxins
3 17, 33F2, 52A1, 63B, and 69D1.

1 18. The nematode toxin, according to claim 1, wherein said toxin is 63B.

1 19. A nucleotide sequence encoding a nematode toxin as defined in claim 1.

1 20. The nucleotide sequence, according to claim 19, which encodes 63B.

1 21. A host comprising a nucleotide sequence which codes for a nematode toxin as defined
2 in claim 1.

1 22. The host, according to claim 21, which is a *Bacillus thuringiensis*.

1 23. The host, according to claim 22, wherein said *Bacillus thuringiensis* comprises
2 inclusions which remain attached to the spore after cell lysis.

1 24. The host, according to claim 23, wherein said *Bacillus thuringiensis* inclusions are long
2 and amorphous.

1 25. The host, according to claim 22, wherein said host has the characteristics of *Bacillus*
2 *thuringiensis* PS63B.

1 26. The host, according to claim 21, wherein said nucleotide sequence is a heterologous
2 sequence which has been transformed into said host and wherein said heterologous sequence is
3 expressed at sufficient levels to result in the production of said nematode toxin.

1 27. The host, according to claim 26, wherein said host is capable of inhabiting the
2 phylloplane or rhizosphere of a plant.

1 28. The host, according to claim 26, which is transformed with a nucleotide sequence
2 which codes for 63B.

1 29. A process for controlling nematodes, wherein said process comprises contacting said
2 nematodes with a nematode-controlling effective amount of a toxin as defined in claim 1.

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1 30. A nematicidal composition comprising substantially intact cells which express a toxin
2 as defined in claim 1.

1 31. The nematicidal composition, according to claim 30, wherein said cells have been
2 treated to prolong their nematicidal activity.

INTERNATIONAL SEARCH REPORT

International App. No. PCT/US 92/03624

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl.5 C 12 N 15/32 C 12 P 21/00 C 12 N 1/20 A 01 N 63/00 //(C 12 P 21/00 C 12 R 1:07)		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl.5	C 07 K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	EP,A,0303426 (MYCOGEN CORP.) 15 February 1989, see the whole document (cited in the application) ---	2-31
A	EP,A,0352052 (MYCOGEN CORP.) 24 January 1990, see the whole document ---	2-31
A	EP,A,0195285 (UNIVERSITY OF GEORGIA RESEARCH) 24 September 1986, see abstract; claim 30 ---	1,9,21-31
A	EP,A,0063949 (THE BOARD OF REGENTS OF THE UNIV. OF WASHINGTON) 3 November 1982, see claims 1-10 ---	1,17,21-31
A	WO,A,9008821 (UNIV. OF MIAMI) 9 August 1990, see abstract; claim 1 -----	1,10-16
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
23-07-1992	07 SEP 1992	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	D GURDJIAN	

INTERNATIONAL SEARCH REPORT

International application No.

US 92/ 03624

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1-31 (partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
See Art. 84 of EPC: claims shall be clear and concise and supported by the description
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9203624
SA 60883

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 07/08/92. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0303426	15-02-89	US-A- 4948734	14-08-90
		JP-A- 1067192	13-03-89
		US-A- 5093120	03-03-92
EP-A- 0352052	24-01-90	JP-A- 2073005	13-03-90
		JP-A- 3024005	01-02-91
EP-A- 0195285	24-09-86	AU-B- 591477	07-12-89
		AU-A- 5416486	04-09-86
		JP-A- 61274685	04-12-86
EP-A- 0063949	03-11-82	US-A- 4448885	15-05-84
		US-A- 4467036	21-08-84
		AU-B- 550672	27-03-86
		AU-A- 8458382	24-11-82
		CA-A- 1198992	07-01-86
		WO-A- 8203872	11-11-82
WO-A- 9008821	09-08-90	EP-A- 0456721	21-11-91

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